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P. ARIASI POLYPEPTIDES, P. PERNICIOSUS POLYPEPTIDES AND METHODS OF USE

PRIORITY CLAIM

This application claims the benefit of U.S. Provisional Application No. 60/412,327, filed September 19, 2002, and U.S. Provisional Application No. 60/425,852, filed November 12, 2002, which are incorporated herein by reference.

FIELD

The disclosure relates to proteins substantially purified from Phlebotomine sand fly salivary glands, or recombinant vectors expressing these proteins, and to an immune response produced to these proteins. This disclosure also relates to the production of an immune response that affects survival of Leishmania.

BACKGROUND

Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania* that affects many millions of people worldwide. In humans, infection with the parasite manifests either as a cutaneous disease caused mainly by *L. major, L. tropica*, and *L. mexicana*; as a mucocutaneous disease caused mainly by *L. brasiliensis*; or as a visceral disease caused mainly by *L. donovani* and *L. chagasi*. In canids, Leishmania infections manifest as a visceral disease that can result in high death rates.

All leishmanial diseases are transmitted to their vertebrate hosts by phlebotomine sand flies, which acquire the pathogen by feeding on infected hosts and transmit them by regurgitating the parasite at the site of a subsequent blood meal (Killick-Kendrick, Biology of *Leishmania* in phlebotomine sand flies. *In* Biology of the kinetoplastida. W. Lumsden and D. Evans, editors. Academic Press, New York. 395, 1979).

While obtaining a blood meal, sand flies salivate into the host's skin. This saliva contains anticlotting, antiplatelet, and vasodilatory compounds that increase the hemorrhagic pool where sand flies feed (Ribeiro *et al., Comp. Biochem. Physiol.* 4:683, 1986; Charlab *et al., Proc. Natl. Acad. Sci. USA.* 26:15155, 1999). Some of

these components are additionally immunomodulatory. For example, the New World sand fly *Lutzomyia longipalpis* contains the 6.5 kDa peptide, maxadilan, which is the most potent vasodilator known (Lerner *et al., J. Biol. Chem.* 17:11234, 1991). Maxadilan additionally has immunosuppressive activities of its own (Qureshi et al., *Am. J. Trop. Med. Hyg.* 6:665, 1996), as do many persistent vasodilators such as prostaglandin E_2 (Makoul *et al., J. Immunol.* 134:2645, 1985; Santoli and Zurier, *J. Immunol.* 143:1303, 1989; Stockman and Mumford, *Exp. Hematol.* 2:65, 1974) and calcitonin gene-related peptide (Nong *et al., J. Immunol.* 1:45, 1989). Old World sand flies do not have maxadilan but instead use adenosine monophosphate and adenosine as vasodilators (Ribeiro *et al., J. Exp. Biol.* 11:1551, 1999). Adenosine is also an immunomodulatory component, promoting the production of interleukin-10 and suppressing tumor necrosis factor-α and interleukin-12 in mice (Hasko *et al., J. Immunol.* 10:4634, 1996; Webster, *Asian Pac. J. Allergy Immunol.* 2:311, 1984; Hasko *et al., FASEB J.* 14:2065, 2000). Despite what is known about the role of sandfly saliva and disease transmission, much remains unknown, and an effective vaccine does not exist. Thus, there is a need for agents that can be used to induce an immune response to the organisms that cause leishmaniasis.

SUMMARY

The present disclosure relates to salivary proteins from sand fly vectors of *Leishmania* that are members of the subgenus of Phlebotomus Larroussius, in particular two species, namely *Phlebotomus ariasi* and *Phlebotomus perniciosus*, and the nucleic acids that encode these proteins. Methods of producing an immune response in a subject are also disclosed.

Substantially purified salivary *P. ariasi* polypeptides are disclosed herein. Also disclosed are polynucleotides encoding the *P. ariasi* polypeptides disclosed herein.

Disclosed herein are substantially purified salivary *P. perniciosus* polypeptides. Also disclosed are polynucleotides encoding the *P. perniciosus* polypeptides disclosed herein.

Methods are disclosed for inducing an immune response to a *P. ariasi* polypeptide using a therapeutically effective amount of the substantially purified salivary *P. ariasi* polypeptides disclosed herein, or the polynucleotides encoding the *P. ariasi* polypeptides disclosed herein.

Methods are also disclosed for inducing an immune response to a *P. perniciosus* polypeptide using a therapeutically effective amount of the *P. perniciosus* polypeptides disclosed herein, or the polynucleotides encoding the *P. perniciosus* polypeptides disclosed herein.

In another embodiment, methods are disclosed herein for inhibiting the symptoms of a *Leishmania* infection or for preventing a *Leishmania* infection in a subject. The methods include administering to the subject a therapeutically effective amount of a *P. ariasi* polypeptide, or a polynucleotide encoding a *P. ariasi* polypeptide.

In yet another embodiment, methods are disclosed herein for inhibiting the symptoms of a *Leishmania* infection or for preventing a *Leishmania* infection in a subject. The methods include administering to the subject a therapeutically effective amount of a *P. perniciosus* polypeptide, or a polynucleotide encoding a *P. perniciosus* polypeptide.

Pharmaceutical compositions are disclosed including a pharmaceutically acceptable carrier and a *P. ariasi* polypeptide and/or a *P. perniciosus* polypeptide.

The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the accompanying sequence listing:

SEQ ID NO: 1 is the amino acid sequence of a PRL-P4-A10 polypeptide.

SEQ ID NO:2 is the nucleic acid sequence of a polynucleotide encoding a PRL-P4-A10 polypeptide.

SEQ ID NO: 3 is the amino acid sequence of PRL-P4-A9 polypeptide.

SEQ ID NO:4 is the nucleic acid sequence of a polynucleotide encoding a PRL-P4-A9 polypeptide.

SEQ ID NO:5 is the amino acid sequence of PRL-P4-C10 polypeptide.

SEQ ID NO:6 is the nucleic acid sequence of a polynucleotide encoding a PRL-P4-C10 polypeptide.

SEQ ID NO:7 is the amino acid sequence of PRL-P4-D6 polypeptide.

SEQ ID NO:8 is the nucleic acid sequence of a polynucleotide encoding a PRL-P4-D6 polypeptide.

SEQ ID NO:9 is the amino acid sequence of PRL-P4-D7 polypeptide.

SEQ ID NO:10 is the nucleic acid sequence of a polynucleotide encoding a PRL-P4-D7 polypeptide.

SEQ ID NO:11 is the amino acid sequence of PRL-P4-E5 polypeptide.

SEQ ID NO:12 is the nucleic acid sequence of a polynucleotide encoding a PRL-P4-E5 polypeptide.

SEQ ID NO:13 is the amino acid sequence of PRL-P4-F3 polypeptide.

SEQ ID NO:14 is the nucleic acid sequence of a polynucleotide encoding a PRL-P4-F3 polypeptide.

SEQ ID NO:15 is the amino acid sequence of PRL-P4-G12 polypeptide.

SEQ ID NO:16 is the nucleic acid sequence of a polynucleotide encoding a PRL-P4-G12 polypeptide.

SEQ ID NO:17 is the amino acid sequence of PRL-P4-G7 polypeptide.

SEQ ID NO:18 is the nucleic acid sequence of a polynucleotide encoding a PRL-P4-G7 polypeptide.

SEQ ID NO:19 is the amino acid sequence of PRL-P6-E11 polypeptide.

SEQ ID NO:20 is the nucleic acid sequence of a polynucleotide encoding a PRL-P6-E11 polypeptide.

SEQ ID NO:21 is the amino acid sequence of PRM-P3-A6 polypeptide.

SEQ ID NO:22 is the nucleic acid sequence of a polynucleotide encoding a PRM-P3-A6 polypeptide.

SEQ ID NO:23 is the amino acid sequence of PRM-P3-F11 polypeptide.

SEQ ID NO:24 is the nucleic acid sequence of a polynucleotide encoding a PRM-P3-F11 polypeptide.

SEQ ID NO:25 is the amino acid sequence of PRM-P5-D6 polypeptide.

SEQ ID NO:26 is the nucleic acid sequence of a polynucleotide encoding a PRM-P5-D6 polypeptide.

SEQ ID NO:27 is the amino acid sequence of PRM-P5-E9 polypeptide.

SEQ ID NO:28 is the nucleic acid sequence of a polynucleotide encoding a PRM-P5-E9 polypeptide.

SEQ ID NO:29 is the amino acid sequence of PRM-P5-F12 polypeptide.

SEQ ID NO:30 is the nucleic acid sequence of a polynucleotide encoding a PRM-P5-F12 polypeptide.

SEQ ID NO:31 is the amino acid sequence of PRM-P5-F2 polypeptide.

SEQ ID NO:32 is the nucleic acid sequence of a polynucleotide encoding a PRM-P5-F2 polypeptide.

SEQ ID NO:33 is the amino acid sequence of PRM-P5-G11 polypeptide.

SEQ ID NO:34 is the nucleic acid sequence of a polynucleotide encoding a PRM-P5-G11 polypeptide.

SEQ ID NO:35 is the amino acid sequence of PRM-P5-H4 polypeptide.

SEQ ID NO:36 is the nucleic acid sequence of a polynucleotide encoding a PRM-P5-H4 polypeptide.

SEQ ID NO:37 is the amino acid sequence of PRS-P1-B11 polypeptide.

SEQ ID NO:38 is the nucleic acid sequence of a polynucleotide encoding a PRS-P1-B11 polypeptide.

SEQ ID NO:39 is the amino acid sequence of PRS-P1-B4 polypeptide.

SEQ ID NO:40 is the nucleic acid sequence of a polynucleotide encoding a PRS-P1-B4 polypeptide.

SEQ ID NO:41 is the amino acid sequence of PRS-P1-E7 polypeptide.

SEQ ID NO:42 is the nucleic acid sequence of a polynucleotide encoding a PRS-P1-E7 polypeptide.

SEQ ID NO:43 is the amino acid sequence of PRS-P1-G9 polypeptide.

SEQ ID NO:44 is the nucleic acid sequence of a polynucleotide encoding a PRS-P1-G9 polypeptide.

SEQ ID NO:45 is the amino acid sequence of PRS-P2-C8 polypeptide.

SEQ ID NO:46 is the nucleic acid sequence of a polynucleotide encoding a PRS-P2-C8 polypeptide.

SEQ ID NO:47 is the amino acid sequence of PRS-P2-G8 polypeptide.

SEQ ID NO:48 is the nucleic acid sequence of a polynucleotide encoding a PRS-P2-G8 polypeptide.

SEQ ID NO:49 is the amino acid sequence of PERL-P7-G8 polypeptide.

SEQ ID NO:50 is the nucleic acid sequence of a polynucleotide encoding a PERL-P7-G8 polypeptide.

SEQ ID NO:51 is the amino acid sequence of PERL-P6-H9 polypeptide.

SEQ ID NO:52 is the nucleic acid sequence of a polynucleotide encoding a PERL-P6-H9 polypeptide.

SEQ ID NO:53 is the amino acid sequence of PERL-P7-C2 polypeptide.

SEQ ID NO:54 is the nucleic acid sequence of a polynucleotide encoding a PERL-P7-C2 polypeptide.

SEQ ID NO:55 is the amino acid sequence of PERL-P6-H1 polypeptide.

SEQ ID NO:56 is the nucleic acid sequence of a polynucleotide encoding a PERL-P6-H1 polypeptide.

SEQ ID NO:57 is the amino acid sequence of PERL-P3-E11 polypeptide.

SEQ ID NO:58 is the nucleic acid sequence of a polynucleotide encoding a PERL-P3-E11polypeptide.

SEQ ID NO:59 is the amino acid sequence of PERL-P7-G12 polypeptide.

SEQ ID NO:60 is the nucleic acid sequence of a polynucleotide encoding a PERL-P7-G12 polypeptide.

SEQ ID NO:61 is the amino acid sequence of PERL-P3-C9 polypeptide.

SEQ ID NO:62 is the nucleic acid sequence of a polynucleotide encoding a PERL-P3-C9 polypeptide.

SEQ ID NO:63 is the amino acid sequence of PERM-P2-A10 polypeptide.

SEQ ID NO:64 is the nucleic acid sequence of a polynucleotide encoding a PERM-P2-A10 polypeptide.

SEQ ID NO:65 is the amino acid sequence of PERL-P6-H11 polypeptide.

SEQ ID NO:66 is the nucleic acid sequence of a polynucleotide encoding a PERL-P6-H11 polypeptide.

SEQ ID NO:67 is the amino acid sequence of PERS-P1-H11 polypeptide.

SEQ ID NO:68 is the nucleic acid sequence of a polynucleotide encoding a PERS-P1-H11 polypeptide.

SEQ ID NO:69 is the amino acid sequence of PERM-P2-G11 polypeptide.

SEQ ID NO:70 is the nucleic acid sequence of a polynucleotide encoding a PERM-P2-G11 polypeptide.

SEQ ID NO:71 is the amino acid sequence of PERM-P5-E2 polypeptide.

SEQ ID NO:72 is the nucleic acid sequence of a polynucleotide encoding a PERM-P5-E2 polypeptide.

SEQ ID NO:73 is the amino acid sequence of PERM-P5-C11 polypeptide.

SEQ ID NO:74 is the nucleic acid sequence of a polynucleotide encoding a PERM-P5-C11 polypeptide.

SEQ ID NO:75 is the amino acid sequence of PERM-P5-H8 (also referred to as P2-G9) polypeptide.

SEQ ID NO:76 is the nucleic acid sequence of a polynucleotide encoding a PERM-P5-H8 (also referred to as P2-G9) polypeptide.

SEQ ID NO:77 is the amino acid sequence of PERL-P3-B3 polypeptide.

SEQ ID NO:78 is the nucleic acid sequence of a polynucleotide encoding a PERL-P3-B3 polypeptide.

SEQ ID NO:79 is the amino acid sequence of PERM-P2-D11 polypeptide.

SEQ ID NO:80 is the nucleic acid sequence of a polynucleotide encoding a PERM-P2-D11 polypeptide.

SEQ ID NO:81 is the amino acid sequence of PERM-P5-E3 polypeptide.

SEQ ID NO:82 is the nucleic acid sequence of a polynucleotide encoding a PERM-P5-E3 polypeptide.

SEQ ID NO:83 is the amino acid sequence of PERM-P2-F11 polypeptide.

SEQ ID NO:84 is the nucleic acid sequence of a polynucleotide encoding a PERM-P2-F11 polypeptide.

SEQ ID NO:85 is the nucleic acid sequence of the PT2F1 primer.

SEQ ID NO:86 is the nucleic acid sequence of the PT2R1 primer.

SEQ ID NO:87 is the nucleic acid sequence of the PT2F3 primer.

DETAILED DESCRIPTION

I. Abbreviations

AAV	adeno-associated virus
AcNPV	Autographa California Nuclear Polyhedrosis Virus
alum	aluminum phosphate or aluminum hydroxide
BCG	Bacillus Calmette Guerin
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumin
CAV	canine adenovirus
CDR	complementarity determining region
CHV	canine herpes virus
CMV	cytomegalovirus
CTL	cytotoxic T lymphocyte
DMRIE	N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanammonium
DOPE	dioleoyl-phosphatidyl-ethanolamine
DTH	delayed type hypersensitivity
fMLP	N-formyl-methionyl-leucyl-phenylalanine
GM-CSF	granulocyte-macrophage colony stimulating factor
H	heavy chains
HLB	hydrophile-lipophile balance
ID	intradermal
IM	intramuscular
ISS	immunostimulating sequence
KLH	keyhole limpet hemocyanin
L	light chains
LB	Luria broth
MVA	Modified Vaccinia virus Ankara
ORF	open reading frame
PCR	polymerase chain reaction
polyA	polyadenylation signal
PVDF	polyvinylidene difluoride
SC	subcutaneous
SCA	Single chain antibody
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sFv	single-chain antigen binding proteins
SGH	salivary gland homogenate

SPGA	sucrose phosphate glutamate albumin
tPA	tissue plasminogen activator
V_H	variable region of the heavy chain
V_L	variable region of the light chain
W/V	weight/volume

II. Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

Amplification (of a nucleic acid molecule): A technique that increases the number of copies of a nucleic acid molecule (e.g., a DNA or an RNA) in a specimen. An example of amplification is the polymerase chain reaction, in which a biological sample collected from a subject is contacted with a pair of oligonucleotide primers, under conditions that allow for the hybridization of the primers to a nucleic acid template in the sample. The primers are extended under suitable conditions, dissociated from the template, and then re-annealed, extended, and dissociated to amplify the number of copies of the nucleic acid. The product of amplification may be characterized by electrophoresis, restriction endonuclease cleavage patterns, oligonucleotide hybridization or ligation, and/or nucleic acid sequencing using standard techniques. Other examples of amplification include strand displacement amplification, as disclosed in U.S. Patent No. 5,744,311; transcription-free isothermal amplification, as disclosed in U.S. Patent No. 6,033,881; repair chain reaction amplification, as disclosed in WO 90/01069; ligase chain reaction amplification, as disclosed in EP 0320308; gap filling ligase chain reaction amplification, as disclosed in 5,427,930; and NASBA™ RNA transcription-free amplification, as disclosed in U.S. Patent No. 6,025,134.

Antibody: immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen.

A naturally occurring antibody (*e.g.*, IgG, IgM, IgD) includes four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. However, it has been shown that the antigen-binding function of an antibody can be performed by fragments of a naturally occurring antibody. Thus, these antigen-binding fragments are also intended to be designated by the term "antibody." Specific, non-limiting examples of binding fragments encompassed within the term antibody include (i) a Fab fragment consisting of the VL, VH, CL, and CH1 domains; (ii) an Fd fragment consisting of the VH and CH1 domains; (iii) an Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (iv) a dAb fragment (Ward *et al.*, *Nature* **341**:544-546, 1989) which consists of a VH domain; (v) an isolated complimentarity determining region (CDR); and (vi) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region.

Immunoglobulins and certain variants thereof are known and many have been prepared in recombinant cell culture (*e.g., see* U.S. Patent No. 4,745,055; U.S. Patent No. 4,444,487; WO 88/03565; EP 0256654; EP 0120694; EP 0125023; Faoulkner *et al.*, *Nature* **298**:286, 1982; Morrison, *J. Immunol.* **123**:793, 1979; Morrison *et al.*, *Ann Rev. Immunol* **2**:239, 1984).

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects, such as dogs.

Conservative variants: "Conservative" amino acid substitutions are those substitutions that do not substantially affect or decrease an activity or antigenicity of the *P. ariasi* or *P. perniciosus* polypeptide. Specific, non-limiting examples of a conservative substitution include the following examples:

Original Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid, provided that antibodies raised to the unsubstituted polypeptide also essentially immunoreact with the substituted polypeptide, or that an immune response can be generated against the substituted polypeptide that is similar to the immune response against the unsubstituted polypeptide. Thus, in one embodiment, non-conservative substitutions are those that reduce an activity or antigenicity.

cDNA (complementary DNA): A piece of DNA lacking internal, non-coding segments (introns) and expression control sequences. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

Degenerate variant: A polynucleotide encoding a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide that includes a sequence that is degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the *P. ariasi* polypeptide or *P. perniciosus* polypeptide encoded by the nucleotide sequence is unchanged.

Epitope: An antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, *i.e.*, that elicit a specific immune response. An antibody specifically binds a particular antigenic epitope on a polypeptide. Specific, non-limiting examples of an epitope include a tetra- to penta-peptide sequence in a polypeptide, a tri- to penta-glycoside sequence in a polysaccharide. In the animal most antigens will present several or even many antigenic determinants simultaneously. Such a polypeptide may also be qualified as an immunogenic polypeptide and the epitope may be identified as described further.

Expression Control Sequences: Nucleic acid sequences that control and regulate the expression of a nucleic acid sequence, such as a heterologous nucleic acid sequence, to which it is operably linked. Expression control sequences are operably linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, polyA signals, a start codon (*i.e.*, ATG) in front of a protein-encoding polynucleotide sequence, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters, are included (see *e.g.*, Bitter *et al.*, *Methods in Enzymology* 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac-hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian

viruses (*e.g.*, the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences. In one embodiment, the promoter is a cytomegalovirus promoter.

Host cells: Cells in which a vector can be propagated and its DNA expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Also includes the cells of the subject.

Immune response: A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular antigen (an "antigen-specific response"). The response can also be a non-specific response (not targeted specifically to salivary polypeptides) such as production of lymphokines. In one embodiment, an immune response is a T cell response, such as a CD4+ response or a CD8+ response. In another embodiment, the response is a B cell response, and results in the production of specific antibodies.

Immunogenic polypeptide: A polypeptide which comprises an allele-specific motif, an epitope or other sequence such that the polypeptide will induce an immune response. A specific, non-limiting example of an immune response includes binding an MHC molecule and inducing a cytotoxic T lymphocyte ("CTL") response, inducing a B cell response (*e.g.*, antibody production), and/or T-helper lymphocyte response, and/or a delayed type hypersensitivity (DTH) response against the antigen from which the immunogenic polypeptide is derived.

In one embodiment, immunogenic polypeptides are identified using sequence motifs or other methods known in the art. Typically, algorithms are used to determine the "binding threshold" of polypeptides to select those with scores that give them a high probability of binding at a certain affinity and will be immunogenic. The algorithms are based either on the effects on MHC binding of a particular amino acid at a particular position, the effects on antibody binding of a particular amino acid at a particular position, or the effects on binding of a particular

substitution in a motif- containing polypeptide. Within the context of an immunogenic polypeptide, a "conserved residue" is one which appears in a significantly higher frequency than would be expected by random distribution at a particular position in a polypeptide. In one embodiment, a conserved residue is one where the MHC structure may provide a contact point with the immunogenic polypeptide.

Immunogenic composition: A composition that, when administered to a subject, induces an immune response to a Phlebotomus salivary polypeptide. In one embodiment, the immune response is a positive DTH response.

Isolated: An "isolated" biological component (such as a nucleic acid or protein or organelle) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, *i.e.*, other chromosomal and extra-chromosomal DNA and RNA, proteins, and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant technology as well as chemical synthesis.

Label: A detectable compound or composition that is conjugated directly or indirectly to another molecule to facilitate detection of that molecule. Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, and radioactive isotopes.

Leishmaniasis: A parasitic disease spread by the bite of infected sand flies. The trypanosomatid parasite of the genus *Leishmania* is the etiological agent of a variety of disease manifestations, which are collectively known as leishmaniasis. Leishmaniasis is prevalent through out the tropical and sub-tropical regions of Africa, Asia, the Mediterranean, Southern Europe (old world), and South and Central America (new world). The old world species are transmitted by the sand fly vector *Phlebotomus sp.* Humans, wild animals and domestic animals (such as dogs) are known to be targets of these sandflies and to act as reservoir hosts or to develop leishmaniasis.

Cutaneous leishmaniasis starts as single or multiple nodules that develop into ulcers in the skin at the site of the bite. The chiclero ulcer typically appears as a

notch-like loss of tissue on the ear lobe. The incubation period ranges from days to months, even a year in some cases. The sores usually last months to a few years, with most cases healing on their own. The mucocutaneous type can develop into erosive lesions in the nose, mouth, or throat and can lead to severe disfigurement. Visceral leishmaniasis often has fever occurring in a typical daily pattern, abdominal enlargement with pain, weakness, widespread swelling of lymph nodes, and weight loss, as well as superimposed infections because of a weakened immune system. Visceral leishmaniasis can result in high death rates. The onset of symptoms can be sudden, but more often tends to be insidious.

Lymphocytes: A type of white blood cell that is involved in the immune defenses of the body. There are two main types of lymphocytes: B cells and T cells.

Mammal: This term includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

Oligonucleotide: A linear polynucleotide sequence of up to about 100 nucleotide bases in length.

Open reading frame (ORF): A nucleic acid sequence having a series of nucleotide triplets (codons), starting with a start codon and ending with a stop codon, coding for amino acids without any internal termination codons. These sequences are usually translatable into a polypeptide.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Pharmaceutically acceptable vehicles or excipients: The pharmaceutically acceptable vehicles or excipients of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the polypeptides, plasmids, viral vectors herein disclosed.

In general, the nature of the vehicle or excipient will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (*e.g.*, freeze-dried pastille, powder, pill, tablet, or capsule forms), conventional non-toxic solid vehicles or excipients can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral vehicles or excipients, immunogenic compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Phlebotomus ariasi (P. ariasi): A species of Phlebotomus (sand flies) genus endogenous to the Old World, in particular to southern Europe and Mediterranean countries, more particularly to Spain and France. This sand fly is a proven vector of visceral leishmaniasis. *P. ariasi* is a member of the subgenera of Phlebotomus Larroussius.

***Phlebotomus perniciosus* (*P. perniciosus*):** A species of Phlebotomus (sand flies) genus endogenous to the Old World, in particular to southern Europe, and Mediterranean countries, more particularly to France, Italy, Greece, Morocco, and Spain. This sand fly is a proven vector of the visceral leishmaniasis. *P. perniciosus* is a member of the subgenera of Phlebotomus Larroussius.

Polynucleotide: The term polynucleotide or nucleic acid sequence refers to a polymeric form of nucleotide at least 10 bases in length, thus including oligonucleotides and genes. A recombinant polynucleotide includes a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA) independent of other sequences. The polynucleotides can be ribonucleotides (e.g.

RNA), deoxyribonucleotides (e.g. DNA, cDNA), or modified forms of either nucleotide. The term includes single- and double-stranded forms of DNA.

Polypeptide: Any chain of amino acids, regardless of length (thus encompassing oligopeptides, peptides, and proteins) or post-translational modification (*e.g.*, glycosylation, phosphorylation, or acylation). A polypeptide encompasses also the precursor, as well as the mature polypeptide. In one embodiment, the polypeptide is a polypeptide isolated from *P. ariasi*, or encoded by a nucleic acid isolated from *P. ariasi*, such as the *P. ariasi* polypeptides disclosed herein. In another embodiment, the polypeptide is a polypeptide isolated from *P. perniciosus*, or encoded by a nucleic acid isolated from *P. perniciosus*, such as the *P. perniciosus* polypeptides disclosed herein.

Fusion proteins are encompassed by the term polypeptide. Fusion proteins have at least two domains of two different polypeptides fused together. In one embodiment, one domain is a detectable label. The two domains of a fusion protein can be genetically fused together, for instance directly or through the use of a linker oligonucleotide, thereby producing a single fusion-encoding nucleic acid molecule. The translated product of such a fusion-encoding nucleic acid molecule is a fusion protein. In one embodiment, one domain of the fusion protein is a *P. ariasi* or a *P. perniciosus* polypeptide and another domain of the fusion protein is a detectable label. The detectable label can be green fluorescent protein, a myc tag or a histidine tag, or the like.

Polypeptide Modifications: *P. ariasi* polypeptides or *P. perniciosus* polypeptides include synthetic embodiments of polypeptides described herein. In addition, analogues (non-peptide organic molecules), derivatives (chemically functionalized peptide molecules obtained starting with the disclosed polypeptide sequences) and variants (homologs) of these proteins can be utilized in the methods described herein. Each polypeptide of the disclosure is comprised of a sequence of amino acids, which may be either L- and/or D- amino acids, naturally occurring and otherwise.

Polypeptides may be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified polypeptides, and optionally having other desirable properties. For example,

carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C_1-C_{16} ester, or converted to an amide of formula NR_1R_2 wherein R_1 and R_2 are each independently H or C_1-C_{16} alkyl, or combined to form a heterocyclic ring, such as a 5- or 6- membered ring. Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric, and other organic salts, or may be modified to C_1-C_{16} alkyl or dialkyl amino or further converted to an amide.

Hydroxyl groups of the peptide side chains may be converted to C_1-C_{16} alkoxy or to a C_1-C_{16} ester using well-recognized techniques. Phenyl and phenolic rings of the peptide side chains may be substituted with one or more halogen atoms, such as fluorine, chlorine, bromine, or iodine, or with C_1-C_{16} alkyl, C_1-C_{16} alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the peptide side chains can be extended to homologous C_2-C_4 alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the peptides of this disclosure to select and provide conformational constraints to the structure that result in enhanced stability.

Peptidomimetic and organomimetic embodiments are envisioned, whereby the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the peptide backbone and component amino acid side chains, resulting in such peptido- and organomimetics of a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide having measurable or enhanced ability to generate an immune response. For computer modeling applications, a pharmacophore is an idealized, three-dimensional definition of the structural requirements for biological activity. Peptido- and organomimetics can be designed to fit each pharmacophore with current computer modeling software (using computer assisted drug design or CADD). See Walters, "Computer-Assisted Modeling of Drugs," Klegerman & Groves (eds.), 1993, *Pharmaceutical Biotechnology*, Interpharm Press: Buffalo Grove, IL, pp. 165-174

and *Principles of Pharmacology* Munson (ed.) 1995, Ch. 102, for descriptions of techniques used in CADD. Also included are mimetics prepared using such techniques.

Probes and primers: A probe comprises an isolated polynucleotide attached to a detectable label or reporter molecule. Primers are short polynucleotides. In one embodiment, polynucleotides are 15 nucleotides or more in length. Primers may be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, *e.g.*, by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art. One of skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, for example, a primer comprising 20 consecutive nucleotides will anneal to a target with a higher specificity than a corresponding primer of only 15 nucleotides. Thus, in order to obtain greater specificity, probes and primers may be selected that comprise at least 15, 20, 25, 30, 35, 40, 50 or more consecutive nucleotides.

Promoter: A promoter is an array of nucleic acid control sequences that directs transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. Both constitutive and inducible promoters are included (see *e.g.*, Bitter *et al.*, *Methods in Enzymology* **153**:516-544, 1987). Promoters may be cell-type specific or tissue specific.

Specific, non-limiting examples of promoters include promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used. A polynucleotide can be inserted into an expression vector that contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host.

The expression vector typically contains an origin of replication, a promoter, as well as specific nucleic acid sequences that allow phenotypic selection of the transformed cells.

Protein Purification: The *P. ariasi* polypeptides and *P. perniciosus* polypeptides disclosed herein can be purified by any of the means known in the art. See, *e.g.*, *Guide to Protein Purification*, Deutscher (ed.), *Meth. Enzymol.* 185, Academic Press, San Diego, 1990; and Scopes, *Protein Purification: Principles and Practice*, Springer Verlag, New York, 1982. Substantial purification denotes purification from other proteins or cellular components. A substantially purified protein is at least 60%, 70%, 80%, 90%, 95%, or 98% pure. Thus, in one specific, non-limiting example, a substantially purified protein is 90% free of other proteins or cellular components.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified polypeptide preparation is one in which the polypeptide is more enriched than the polypeptide is in its natural environment. A polypeptide preparation is substantially purified such that the polypeptide represents, in several embodiments, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%, of the total polypeptide content of the preparation. The same applies for polynucleotides. The polypeptides disclosed herein can be purified by any of the means known in the art (see, *e.g.*, *Guide to Protein Purification*, Deutscher (ed.), *Meth. Enzymol.* 185, Academic Press, San Diego, 1990; and Scopes, *Protein Purification: Principles and Practice*, Springer Verlag, New York, 1982).

Recombinant: A recombinant polynucleotide is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques.

Selectively hybridize: Hybridization under moderately or highly stringent conditions that excludes non-related nucleotide sequences.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (*e.g.*, GC v. AT content), and nucleic acid type (*e.g.*, RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

A specific, non-limiting example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). One of skill in the art can readily determine variations on these conditions (*e.g.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Washing can be carried out using only one of these conditions, *e.g.*, high stringency conditions, or each of the conditions can be used, *e.g.*, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

Sequence identity: The similarity between amino acid sequences is expressed in terms of the percentage identity between the sequences. The higher the percentage, the more similar the two sequences are. Homologs or variants of a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide will possess a significant degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* **2**:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* **48**:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* **85**:2444, 1988; Higgins and Sharp, *Gene* **73**:237, 1988; Higgins and Sharp, *CABIOS* **5**:151, 1989; Corpet *et al.*, *Nucleic Acids Research* **16**:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci.*

U.S.A. **85**:2444, 1988. Altschul *et al., Nature Genet.*, **6**:119, 1994 presents a detailed consideration of sequence alignment methods and identity calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al., J. Mol. Biol.* **215**:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn, and tblastx. A description of how to determine sequence identity using this program is available on the NCBI website on the internet.

Homologs and variants of a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide are typically characterized by possession of at least 75%, for example at least 80%, sequence identity counted over the full length alignment with the amino acid sequence of the *P. ariasi* polypeptide or the *P. perniciosus* polypeptide using the NCBI Blast 2.0, gapped blastp set to default parameters. The comparison between the sequences is made over the full length alignment with the amino acid sequence given in this present disclosure, employing the Blast 2 sequences function using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1).

When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologues and, variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologues could be obtained that fall outside of the ranges provided.

Specific binding agent: An agent that binds substantially only to a defined target. Thus a *P. ariasi* specific binding agent is an agent that binds substantially to a *P. ariasi* polypeptide. Similarly, a *P. perniciosus* specific binding agent is an agent that binds substantially to a *P. perniciosus* polypeptide.

In one embodiment, the specific binding agent is a monoclonal or polyclonal antibody that specifically binds the *P. ariasi* polypeptide. In another embodiment, the specific binding agent is a monoclonal or polyclonal antibody that specifically binds the *P. perniciosus* polypeptide.

Subject: Living multi-cellular vertebrate organisms, a category that includes both human veterinary subjects, including human and non-human mammals. In one embodiment, the subject is a member of the canine family, such as a dog. In another embodiment, the subject is a human.

T Cell: A white blood cell critical to the immune response. T cells include, but are not limited to, $CD4^+$ T cells and $CD8^+$ T cells. A $CD4^+$ T lymphocyte is an immune cell that carries a marker on its surface known as "cluster of differentiation 4" (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. $CD8^+$ T cells carry the "cluster of differentiation 8" (CD8) marker. In one embodiment, a CD8 T cells is a cytotoxic T lymphocytes. In another embodiment, a CD8 cell is a suppressor T cell.

Therapeutically active polypeptide: An agent, such as a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide, that causes induction of an immune response, as measured by clinical response (for example, increase in a population of immune cells, production of antibody that specifically binds the *P. ariasi* polypeptide or *P. perniciosus* polypeptide, a measurable reduction in symptoms resulting from exposure to *Leishmania*, or protection from infection with *Leishmania*). Therapeutically active molecules can also be made from nucleic acids. Examples of a nucleic acid based therapeutically active molecule is a nucleic acid sequence that encodes *a P. ariasi* polypeptide or a *P. perniciosus* polypeptide, wherein the nucleic acid sequence is operably linked to a control element such as a promoter. Therapeutically active agents can also include organic or other chemical compounds that mimic the effects of the *P. ariasi* polypeptide or the *P. perniciosus* polypeptide.

The terms "therapeutically effective fragment of a *P. ariasi* polypeptide" or "therapeutically effective variant of a *P. ariasi* polypeptide" includes any fragment of the *P. ariasi* polypeptide, or variant of the *P. ariasi* polypeptide, that retains a function of the *P. ariasi* polypeptide, or retains an antigenic epitope of the *P. ariasi* polypeptide, or retains the ability to reduce the symptoms from exposure to *Leishmania,* or to protect from infection with *Leishmania.* The terms "therapeutically effective fragment of a *P. perniciosus* polypeptide" or "therapeutically effective variant of a *P. perniciosus* polypeptide" includes any fragment of the *P. perniciosus* polypeptide, or variant of the *P. perniciosus* peptide, that retains a function of the *P. perniciosus* polypeptide, or retains the ability to reduce the symptoms from exposure to *Leishmania*, or to protect from infection with *Leishmania.*

Thus, in one embodiment, a therapeutically effective amount of a fragment of *P. ariasi* polypeptide or a *P. perniciosus* polypeptide is an amount used to generate an immune response to the polypeptide. In another embodiment, a therapeutically effective amount of a fragment of *P. ariasi* polypeptide or a *P. perniciosus* polypeptide is an amount of use to prevent or treat a *Leishmania* infection in a subject. Treatment refers to a therapeutic intervention that confers resistance to infection with *Leishamania*, or a reduction in the symptoms associated with exposure to *Leishamania*. Specific, non-limiting examples of a polypeptide fragment are the N-terminal half or the C-terminal half of one of the *P. ariasi* polypeptides or the *P. perniciosus* polypeptide disclosed herein.

Transduced: A transduced cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. As used herein, the term transduction encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Vaccine: Composition that when administered to a subject, induces a decrease of the severity of the symptoms of a disorder or disease. In one embodiment, a vaccine decreases the severity of the symptoms of leishmaniasis and/or decreases the parasitic load.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transduced host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. "Comprise" means "include," and a composition that comprises a polypeptide includes that polypeptide. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for polynucleotides or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

P. ariasi and _P. perniciosus_ Polynucleotides and Polypeptides

Salivary polypeptides from sand fly species of the subgenera of Phlebotomus Larroussius, in particular *P. ariasi* and *P. perniciosus,* are disclosed herein. *P. ariasi* polypeptides include polypeptides having a sequence as set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31,

SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, and conservative variants thereof.

Specific, non-limiting examples of an amino acid sequence of a *P. ariasi* polypeptide are set forth below:

PRL-P4-A10 (SEQ ID NO:1)

MKLVPLCILVCFLIIAQQVAQNEASPAKSQDAMYGDWSRWSSCDETCHQTK
VRSCLGAVCERNRLMKERKCPGCGTKVRIVQKLLQLFGMGDSIETDYEDDY
GEHWLTDDRVISSRNDPESAESDELGSFFRDFFHSFDFEWKNPFSNPHENND
VDLEVEEDEEVEELPEIRTSNEEDSVSGADHVCGVTKNERSSGMMAKTIGG
RNSKKGRWPWQVALYNQEYENFFCGGTLISKYWVITAAHCLISDFGSDITIF
SGLYDTGDLVESPYSIHLVRDRVIHPRYDAETNDNDIALLRLYNEVKLSDDV
GIACLPSYSQASPGRSEVCKVLGWGQGTRRTKLQEADMHIQPANSCKRHYY
GTGQLVTRHMLCASSRNYVSDTCGGDSGGPLLCRDTKSPARPWTLFGITSF
GDDCTVSESPGVYARVASFRKWIDSVIECDGSCDN

PRL-P4-A9 (SEQ ID NO:3)

MNILLKVAILVSLCEIGYSWKYPRNADQTLWAWRSCQKGNYDPELVKKW
MAFEIPDDEVTHCYIKCVWTHLGMYDETSQTIRADRVKQQFKARGLSVPAE
ISHLEGSTGGSCVTIYKKTRAFLETQMPNYRIAFYGTVEESDKWFANNPETK
PKRIKISDFCKGREAGTEGTCKHACSMYYYRLVDEDNLVIPFRKLPGILDSQ
LEQCRDQASSETGCKVGDTIYNCLNRINPEGLKKALNTLDEQSLTLY

PRL-P4-C10 (SEQ ID NO:5)

MKIFLCLFAAVSIQGALASQIEREYAWKNIIYEGIDQGSYNIENSIPTAFAHDA
ASKKIFITIPRINQVPITLTEFDSIKYPGGSPPLSKFPGSDNIISVYQPVIDECRRL
WIVDAGQVEYKGDEQKYPKKNPAIIAYDLTKDNYPEIDRYEIPINIAGNPLGF
GGFTVDVTNPKEGCGKTFIYITNFEDNTLIVYDQEKKDSWKISHGSFKPEHES
ILIHNGVDHILKLGIFGITLGDRDSEGNRPAYYLGGSSTKLFEVNTKALKKKE
GEIEPITLGDRGPHSEAIALAYDPKTKVIFFTEYNSKKISCWNIKKPLIHDNMD
KIYASPEFIFGTDISVDSESKLWFFSNGHPPIENLQLSSDKPHIHLISVDTEKAI
RGTKCEVKA

PRL-P4-D6 (SEQ ID NO:7)

MKIFMGLIAVVSLQGALAYHVEREYAWKNITFEGIDQASYNIENSIPTAFVH
DALSKKIIIAIPRLYPQVPITLTQLDTTKHPERSPPLEKFPGSDKLTSVYQPML
DECRRLWIVDVGQVEYKGDEQKYPKKNPAIIAYDLTKDNYPEIDRYEIPINIA
GNQIGFGGFTVDVTNPKEGCGKTFIYITNFEDNTLIVYDQEKKDSWKISHGSF
KPEHESNFSHNGAQYKYKAGIFGITLGDRDPEGNRPAYYLGGSSTKLFEVST
EALKKKGAKFDPVRLGDRGRHTEAIALVYDPKTKVIFFAESDSRQISCWNTQ
KPLNHKNTDVIYASSKFIFGTDIQIDSDSQLWFLSNGQPPIDNLKLTFDKPHIR
LMRVDTKNSIRRTRCEVKPIKKP

PRL-P4-D7 (SEQ ID NO:9)

MFKEIIVVALAVIVAQCAPPAIPIAKQGNDFPVPIVDEKETDDFFDDRFYPDID
DERVGARAPVGGKQTSNRGTSSQSDKVPRPQGSNRGPSSQTTDKVPRPQWP
SRGTNSQNDKVPRPQGSSGQTPPRTPGKVEQSGRTNTKDQIPRPLTNRNPTK
NPTEQARRPGNRELLIRDKTPGSQGGKQGTGNRQKLSSYKDAQPKLIFKSSQ
FNTDGQNPYLTRLFKTKKVEEVIAKGSPTDEYVLELLDGKPDNLSLVIRTNG
KTSQAVLRNPTRNRIVGRIKSYNPGPRRMSY

PRL-P4-E5 (SEQ ID NO:11)

MASIKLSTCSFVLLNLILPTISMKVISFDDRDEYLLGKPANSDDELLYSTFDFQ
RDPCSKSYVKCTNNNTHFILDFVDPKKRCISSIHVFSYPDRPPSFEEKRIPSKS
AIYCQKGGIGKSHCLLVFRKKEPREDALVDIRGIPADQTCSLKERYTSGDPK
KTDAYGMAYQFDRKDDWHIQRTGIKTWKRSGNEIFYRKNGLMNHQIRYLS
KFDKYTVTRELVVKNNAKKFTLEFSNFRQYRISFLDIYWFQESQRNKPRLPY
IYYNGHCLPSNKTCQLVFDTDEPITYAFVKVFSNPDHNEPRLRHEDLGRG

PRL-P4-F3 (SEQ ID NO:13)

MVIYLTQNISRALLTLLPNPEDVRSAADVLESFTDDLKSFYPPPDDVNEEVSE
TESRTKRSLIEQLKESQPLKQIRETVAETTKYLKGFLKTKPSGNQTESSNSTST
KTQSRKRRGLTDFIPVNSLKDAISQATSGAMKAFKPSSENKTSSNPLDFLASL
SDISRDLVQNSIKEVSGNLVSSVALYQVNSKLDAIKQSIGIINQEIDRTKKVQ
QYVMNALQQASNITNSIGEQLKSNNCFAQFINPFKLFEEVITCVKNKIENGLK

IAEETFKNLNQALSVPSDIVSEVSKCSQNQNLNPLTKLLCYLRVPLQLDEEKLLLPIEFARRIREITNYFATMRMDLIQCGIATIQSIGDKVENCAIEAILAVKDTLKG

PRL-P4-G12 (SEQ ID NO:15)

MKQFPVILLTLGLLVVKCRSERPEWKCERDFKKIDQNCFRPCTFAIYHFVDNKFRIARKNIENYKKFLIDYNTVKPEVNDLEKHLLDCWNTIKSIEASSRTEKCEQVNNFERCVIDKNILNYPVYFNALKKINKNTNV

PRL-P4-G7 (SEQ ID NO:17)

MINPIVLRFTFLLVILLPGKCKSAPKSCTINLPTSIPKKGEPIYLNSNGSVFRPIGGLTQLNIGDSLSIYCPPLKKLKSVPCSRKFSLESYSCNNSSQSELVQTEEECGQEGKWYNIGFPLPTNAFHTIYRTCFNKQKLTPIYSYHVINGKAVGYHVKQPRGNFRPGKGVYRKININELYKTHISRFKKVFGDKQTFFRKPLHYLARGHLSPEVDFVFGTEQHATEFYINTAPQYQSINQGNWLRVEKHVRGLAKALQDNLLVVTGILDILKFSNKRADTEIYLGDGIIPVPQIFWKAIFHLRTSSAIVFVTSNNPHETTFNNICKDACEMAGFGDKQHGNQNFSNYSLGFTICCELQDFIGNSKVVLPKDIQVKNHRKLLQLPKPKQ

PRL-P6-E11 (SEQ ID NO:19)

MNALLLCVLLSLSGIGYSWKYPRNADQTLWAYRTCQREGKDPALVSKWMNWVLPDDPETHCYVKCVWTNLGSYDDNTGSIMINTVATQFITRGMKVPAEVNNLSGSTSGSCSDIYKKTIGFFKSQKANIQKAYYGTKEESDNWYSKHPNVKPKGTKISDFCKGREGGTEGTYKHACSMYYYRLVDEDNLVIPFRKLKIPGIPGPKIDECRRKASSKTGCKVADALYKCLKAINGKSFENALKKLDEESSRTY

PRM-P3-A6 (SEQ ID NO:21)

MIRILFPLFILSLGIYQVTCLMCHSCTLDGELESCEDSINETYVVKIEEKECKPAQSCGKVSFTANGTVRIGRGCIRSSSSWKIDCRILAKEVRDEGIAVTHCSLCDTDLCNE

PRM-P3-F11 (SEQ ID NO:23)

MLQIKHFLFFVVLFVVAHSNDYCEPKLCKFNNQVKTHIGCKNDGKFVESTC
PKPNDAQMIDMTEQRKNLFLKIHNRLRDRLARGSVSNFKSAAKMPMLKWD
NELARLAEYNVRTCKFAHDQCRSTKACPYAGQNLGQMLSSPDFLDPNYVIK
NITREWFLEYKWANQGHTDKYMTGSGKNGKAIGHFTAFIHEKSDKVGCAV
AKLTNQQYNMKQYLVACNYCYTNMLKEGIYTTGKPCSQCQGKKCDSVYK
NLCDASEKVDPIPDIFKQSRQQRSRK

PRM-P5-D6 (SEQ ID NO:25)

MIVKSFLGVFLVILLVSVTEQDRGVDGHRRTQDDHDYSELAEYDDEDPHQE
VIDGDEEEHELSGGRRLSHEDEDDDDRHYGHRGEDRENSRGRNGGSRNRGS
EEQSYDPYSHERAPTYSESSEYDHSGDYDNSNYQQHSSTPSSYSNIDHYLHLI
QLHSVPSDLAQYADSYLQHSKNSIRYYASHAKDFEKIRPCLESVVKYSNLLN
DDLAKEYIRCQRKCYLERLNSYTSAISQYTVTTNACINNRLH

PRM-P5-E9 (SEQ ID NO:27)

MIIKLCAIAVACLLTGDGEAAPRATRFIPFAVISDLDKKSIKSDQKSFTSIVRY
GELKDNGERYTLSIKSENLHYFTRYAYNGRGAELSELLYFNNKLYTIDDKTG
IIFEVKHGGDLIPWVILSNGDGNQKNGFKAEWATVKGDKLIVGSTGIPWFEE
KTQSLNTYSLWVKEISKEGEVTNINWKSQYSKVKNAMGIPSSVGFVWHEAV
NWSPRKNLWVFMPRKCTTEYFTSQVEEKTGCNQIITANEDFTQVKAIRIDGP
VQDQAAGFSSFKFIPGTQNNDIFALKTIERNGQTATYGTVINIEGKTLLNEKR
ILDDKYEGVAFFKNPEGII

PRM-P5-F12 (SEQ ID NO:29)

MHFKIIFCSLFIVLLGHMAFAESSESSSSESSSSETSEESSEEVVPSPSPSPKHRP
HFGPHHPHGGRPKPPHPPPPKPEPEPDNGSDGGNQDNSNGQDNSNGNSQND
EQDNSQSGSAKRFRQPAVNIVNLVIPFSTI

PRM-P5-F2 (SEQ ID NO:31)

MFSKIFSLAILALALSTVSSETCSNPQVKGASSYTTTDATIVSQIAFITEFSLEC
SNPGAEKVSLFAEVDGRITPVAVIGDTKYQVSWNEEVKKARSGDYNVRLY

DEEGYGAVRKAQRSGEENNAKPLATVVVRHSGSYTGPWFNSEILASGLIAV
VAYFAFATRSKILS

PRM-P5-G11 (SEQ ID NO:33)
MSNLLTIFGAICFLGVANSLQFPRNPDQTRWAEKTCLKESWAPPNLINKWK
QLEFPSTNLTYCYVKCFVMYLGVYNETTKKFNVDGIRSQFTSQGLRPPNGLE
SLQKTSKGTCKDVFRMSAGLIKKYKLEFVKAFHGDSAEAAKWYIEHKGNV
KAKYQKASEFCKTQKDECRLHCRFYYYRLVDEDFQIFNRKFKIYGISDSQLR
QCRSKASQAKGCKVAKVLKNCLDKIDSEKVKTALKTLDEISANYV

PRM-P5-H4 (SEQ ID NO:35)
MYFTHTLNFLLLVILLIMAGFSQANPEKRPCTNCERPKLSAKTPL

PRS-P1-B11 (SEQ ID NO:37)
MTWVILCVALLVASVVAEGGIDAEGNRTKIEKITAGAGSDGKVVYTEGGSF
PEKLEKEQKSVKKELGELPKPTNATFSPPVKVENKTEEVRNATLPVNATTEA
PKVVNTTASTTTVKLTSTSTTTTTPKPKKPSLTISVEDDPSLLEVPVKVQHPQ
TGGRLDVEEPVAQLSHENILEMPVNHRDYIVPIVVLIFAIPMILGLATVVIRRF
RDYRLTRHYRRMDYLVDGMYNE

PRS-P1-B4 (SEQ ID NO:39)
MKKILLFSVIFVALLITAEAIPGKRARPKAPAVTKGRDVPKPRPGQGGQVPV
EPDFPMENLRSRI

PRS-P1-E7 (SEQ ID NO:41)
MAVKNLHKFLLVVGFVSLIHAAYSAAQHRTYLRITEQEFNSLPFDIVLQAVV
SLIILVYSILQVVGEFREIRAAVDLQAKSWETLGNIPSFYMFNHRGKSLSGQY
EDNIDTSAD

PRS-P1-G9 (SEQ ID NO:43)
MMSRWSKSVKFVCLLLCGGFTFLTTSARAKPTLTFQLPPALTNLPPFVGISRF
VERKMQNEQMKTYTGVRQTNESLVMIYHHDLTIAIVELGPEKSLLGCELIEI

NNDDEGAKVLKELATVNIPLEIDFREMVKLMKQCEKIDYIRKVKRQGAPES
DQTTNRQHQTGYFTGATAGLSILSGILPGTKWCGTGDIARTYHDLGTEATM
DMCCRTHDLCPVKVRSYQQRYNLTNKSIYTKSHCKCDDMLFNCLKRTNTS
ASQFMGTIYFNVVQVPCVLDTDRGYRFRKARTFS

PRS-P2-C8 (SEQ ID NO:45)
MKLLPIILLALTVLIVTCQAEHPGTKCRREFAIEEECINHCEYKHFGFTDDQF
RIKKHHRENFKNAMSHYGAIRKDQEGELDKLLNRCAKKAKESPATSKRDK
CYRIINYYRCVVVDNNLINYSVYVKAVTKINDSINV

PRS-P2-G8 (SEQ ID NO:47)
MKELVVFLTLIVLVVICHAERPSQKCRRELKTEEECILHCEYKHYRFTDDQF
RLNADQRGDFRNIMRRYGAIRVDQESQL
DKHLKKCANKVAKTPATSRKDKCRKISRYYHCAVDNKLFKYNDYANAIIK
YDKTINV

P. perniciosus polypeptides include polypeptides having a sequence as set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, and conservative variants thereof.

Specific, non-limiting examples of an amino acid sequence of a *P. perniciosus* polypeptide are set forth below:

PERL-P7-G8 (SEQ ID NO:49)
MKIFLCLIAVVFLQGVVGFHVEREYAWKNISYEGVDPALFNIDNIIPTGFVHD
AINKKIFIAVPRRSPQIPFTLTELDTTKHPERSPPLSKFPGSDKLINVYQPVIDE
CRRLWIADVGRVDYKGDEQKYPNQNAVLIAYDLTKENYPEIHRYEIPSKIAG
SNTIPFGGFAVDVTNPKEGCGKTFVYITNFEDNTLIVYDQEKKDSWKISHGS
FKPEHDSTLSHDGKQYKYRVGLFGITLGDRDPEGNRPAYYIAGSSTKLFEIST
KILKEKGAKFDPVNLGNRGPHTEAVALVYDPKTKVIFFAESDSRQVSCWNT
QKPLNHKNTDVIFASAKFIYGSDISVDSESQLWFLSTGHPPIPNLKLTFDKPHI
RLMRVDTAKAIRRTRCEVKPRKP

PERL-P6-H9 (SEQ ID NO:51)

MKIFLCLIAVVSLQGVLAYDIEREYAWKNISFEGIDPASYSVKNSIVTGFAHD
ADSKKIFITIPRLNPVPITLTELDTTKHPEGSPPLSKFPGSDKLISVYQPVIDECR
RLWIVDAGQVEYKGDEQKIPKKNAAIIAYDLTKDNYPEIDRYEIPNNVAGNP
LGFGGFAVDVTNPKEGCGKTFVYITNFEDNTLIVYDQEKKDSWKISHDSFKP
EHESILTHNGAQHILKLGIFGITLGDLDEEGNRQAYYLGGSSTKLFRVNTKDL
KKKAGQIEFTPLGDRGSHSEALALAYDPKTKVIFFIEYNSKRISCWNTQKSLN
PDNIDVIYHSPDFIFGTDISMDSESKLWFFSNGHPPIENVQLTFDKPHFRLISM
DTKKSIHGTKCEVKP

PERL-P7-C2 (SEQ ID NO:53)

MFKKFILVALVVVVAQCALPAIPIARQGKDFPVPFVSEDNNPDDYFDDQYYP
DINDAGVGSKAPQGSRKPPNRGTIPPPRGDQVSSGGRTPPGRVGQGTSPTKD
KRARPQINRNPTGTVGQGGSPGTKDKRARPQINRNPTGSGTKPRDRELVIRD
KPPSGSQGGKPGRQVRGPKEDLSRYQNAPAKLIFKSSNINTAGKTPKRCEVV

PERL-P6-H1 (SEQ ID NO:55)

MTYFKISTCCLVLISLILPIICIKVIRFDDRDEYLLGKPDNTDEELLYSTFDFIK
NTCANPKMKCTNNATHFVLDFSDPKKRCISSIHVFSTPDGPVNLEEENKPRS
KSSIYCQVGGIGQSYCLLVFKKKERREDALVDIRGLKTCSLKERYTSGDPKK
TDAYGMAYKFDKNDNWSIKREGVKQWKRSGNEIFYRKNGLMNHQIRYLS
KFDKYTVTREMVVKHRAKKFTMDFSNYGQYRISFLDVYWFQESVKHKPKL
PYIYYNGECLPSNKTCQLVFDADEPITYAFVKVFSNPDHNEPRLRHADLGRG

PERL-P3-E11 (SEQ ID NO:57)

MKLLITIGAVCVLQVVTVSSIFFPIPINIQTGTTSSSSGQPGQQVTTSISFSNVS
NITDMVIYLTQNISRALLTRVPNPDDIKSAADILESFTGSLKYFQTPPDDVDQ
EESETKSRSKRSFTDIFKQSSPLKEIGERIEEIKKKLKGMLKPKPQTPSGNQTD
SSNTTSETQSRKKRALTDFIPMDSLKDAISKTGEVLIPSSASANSSPLDFMSKL
SDIANDLIQNSMKEISENLASSVAMYQVNSQLDAIKQSMDIIKQEIDKTQKIQ
KYVKEALNQAKNATKSLGEKLKSSNCFAQFINPFKLFEKGITCVKNKIDNGL

KIAKDTFKNLQQAMSVPSDIQSEVSKCSQNQQLNPIAKLLCYLRTPLQLDDE
KLLLPFEFTRRIREITNYFATMRMDLIRCGIETIQSIGDKVEDCAREAILAVKD
TLKG

PERL-P7-G12 (SEQ ID NO:59)
MKQLVVFLALIVLIVICHAEPPSKKCRSGLVKDEECILHCEYKYYGFTDDNF
ELDSDLRGHFRTAMRKHGAIRIDQERQLDKHLKKCAQEAKKSEKCRKIIQY
YRCAVNNKLFQYNAYAKAIIALDKTINV

PERL-P3-C9 (SEQ ID NO:61)
MINSTVIQFIFLFVIFLPGKSKSAPKTCEINLPTSIPTKGESIYLLNGNGSVFRPD
GKLTQLNIGDSLSIYCPGQKELKRVPCSPKFSLENITCNSNVHSELVDTEEKC
GKDGKCYNISFPLPTNTFHTIYRTCFNKQKLTPIYSYHVINGKAVGYHVKQP
RGNFRPGKGVYRKININELYKTHISRFKRIIGSTQTFFRKPLHYLARGHLSPEV
DFVFGNEQHATEFYINTAPQYQSINQGNWLRVEKHVRKLAKALQDDLHVV
TGILGILKFSNKRAEREIYLGEGVIPVPQIFWKAVFHPKTSSAIVFVSSNNPHE
KTFNPMCKDVCETARFGGKQHENQNFSNHTVGFTICCELPDFLGNSKVILPK
EFQGKNYRKLLKMPGKP

PERM-P2-A10 (SEQ ID NO:63)
MNNLLTFFGVLCFLGFANSLRFPRDPDQTRWAEKTCLREFSRAPPSLLKKW
QQLDFPNTNLTHCFIKCFTSYLGVYNDTTKKFNVDGIKTQFKSQEIPAPQGLE
TLRKTSKGTCKDIYLMTVDLVKKNKLQFAKAFHGISAEAAKWYTQHKGNV
KGKYQKASEFCKSKDDECRLHCRFYYYRLVDEDYQIFNRNLKINGISNAQL
QQCRNKASQAKGCQVAKVLRQCLKDINPENVKATLKELDEISAK

PERL-P6-H11 (SEQ ID NO:65)
MLQIKHFLFFVVLLVIVHANDYCQPKLCTNGKTVKPHIGCRNNGDFDRSAC
PNDAQMVEMTQQRKELFLKIHNRLRDRFARGSVPNFKSAAKMPMLKWDN
ELAKLAEYNVRTCKFAHDQCRATTACPYAGQNLGQMLSSPDYLDPGYAIK
NITREWFLEYKWADQQRTNTFTGGPGKDGKQIGHFTAFVHEKSDKVGCAV
AKLTNRQFNMKQYLIACNYCYTNMMNEKITAQVPPF

PERS-P1-H11 (SEQ ID NO:67)

MIVKGLLGVFLVILLVCVTEQGVDGYHRANGDYGYSYENRHHVVNGDEEEHEIKHTNSRKFDDDDYLFSHGYAAYDDEDDEDERQGYSRGGGGAGDSSRDPGFYRRGSQEQSYDPHSGQTAPGYSESSEYEHSGDYDNSQNQQYSSTPSNANVNLIDQYLHLIQLHSIPSDLVQYAESYLTHAKNSIRYYAVHAKDFERIRPCLESVTKYFNMLNDDLAREYVRCQRQCYLDRLNSYTTAISQYTVTTNACINNRLN

PERM-P2-G11 (SEQ ID NO:69)

MILKLCAIAVLFFLIGDGEAAPRPTRFIPFAIISDLHRKAMHDEKNRFTSIVKYGQLKYNGEKYTLSIRSENLHYFTKDTYKGTGADMSELIYFNDKLYTLNDETGTIYEVKHGGELIPWITLKNDDGNQKDGFKAKWATVKGNKLIVGSAGMAFLDAKTMNIDRDALWVKEISESGHVTNKYWDSQYKKVRDAMGLVSGFVWHEAVNWSPRKNLWVFMPRKCTNEPYTVRLDKKTGCNQIITANENFNDVRAIHINRAAADPASGFSSFKFIPNTRNNDIFAIKTIERNGQTATYGTVIDINGKTLLPDQRILDDKYEGIAFFKDPKGIK

PERM-P5-E2 (SEQ ID NO:71)

MNTLLKVAVLLSLGGTGYSWQYPRNADQTLWAWRSCQKEHIGDDQALLKKWLKFEIPDDKVTHCFIKCTWIHLGMYDEKTKTIRVDKVKQQFEGRKLPVPAEISKLEGPTDGDCEKIYRKTKAFLDAQMKNYRIAFYGIYDGSDAWFAEHPETKPKKTKISEFCKGREGGKEGTCKHACSMYYYRLVDEDNLVIPFRKLPGISESDLKQCRDAASKKSGCQVADDNLRLS

PERM-P5-C11 (SEQ ID NO:73)

MKYFSLNFLLIVILLIVACSPQLPCLPQDSKKKPSNPRPKLSARSGLSY

PERM-P5-H8 (also referred to as P2-G9) (SEQ ID NO:75)

MKKIVLFSFIFVALVISAKAIETELDDPDDATKGRDVAKAEPGQLGQVPVVPDLNPSNTRKRRNRSRKRRRNLGKRLKKVFA

PERL-P3-B3 (SEQ ID NO:77)

MMSRWSKSVKFVCLLLCGGFTFLTTSARAKPTLTFQLPPALTNLPPFIGISRF
VERKMQNDQMKTYTGVRQTNDSLVMIYHHDLTIAIVELGPEKTLLGCELIEI
NNDDEGAKVLTELATVNIPLQIDFREMVKLMKQCEKIDYMRKVKRQGASE
SDQTTNRQHQTGYFGLGGATAGLSILSGILPGTKWCGTGDIAKTYHDLGTE
ATMDMCCRTHDLCPVKVRSYQQRYNLSNNSIYTKSPCKCDDMLFNCLKRT
NTSASQFMGTIYFNVVQVPCVLDTERGYRFRKARTFS

PERM-P2-D11 (SEQ ID NO:79)

MKQLVVFLALIVLIVICHAKRPSRKCRSGMVKEEECILHCEYKYYGFTDDKF
QLDADQRGNFRFAMMDYGAIRMDQEGQMDEHLKKCANEAEKAPVCSKV
DKCRKIIQYYRCAVNNKLFQYNAYAKAIIALDKTINV

PERM-P5-E3 (SEQ ID NO:81)

MKQLPVILLALVFLIAKCRSEKPEYKCRRDFKTEDKNCFLSCTFKNYHFIDN
KFRIERKNIENYKKFITDYKALKPNVSDNDLEKHLLDCWDKFQKSPEASTRP
EKCEKVNNFERCVIDKNIFDYPIYFNALKKINYITKV

PERM-P2-F11 (SEQ ID NO:83)

MKKIVLFSVIFIALVISAKAIEDEDDDDDDDESEDRDVARAEREQQEEEPDEP
EYIPSRPRNRSKMRKWRNRNYRKYRDESRKRKRDMVLDVIRRFL

Homologous polypeptides having an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence as set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ

ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83 are disclosed herein.

Fragments, variants, and fusions of the *Phlebotomus* polypeptides identified above are disclosed herein and can readily be prepared by one of skill in the art using molecular techniques. In one embodiment, a fragment of a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids of a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide. In another embodiment, a fragment of a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide includes a specific antigenic epitope found on a full-length *P. ariasi* polypeptide or on a full-length *P. perniciosus* polypeptide.

In one embodiment, a fragment is at least 17 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids in length from any polypeptide (including polypeptides as given in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83, conservative variants thereof, and homologues thereof), or any fragment that retains at least an epitope.

One skilled in the art, given the disclosure herein, can purify a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the *P. ariasi* polypeptide or the *P. perniciosus* polypeptide can also be determined by amino-terminal amino acid sequence analysis.

Minor modifications of the *P. ariasi* polypeptide or the *P. perniciosus* polypeptide primary amino acid sequences may result in peptides which have substantially equivalent activity as compared to the unmodified counterpart

polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein.

Polynucleotides encoding salivary polypeptides from sand fly species of the subgenera of Phlebotomus Larroussius, in particular *P. ariasi* and *P. perniciosus*, are disclosed herein. Also provided are nucleic acid sequences encoding a *P. ariasi* or a *P. perniciosus* polypeptide. Specific, non-limiting examples of *P. ariasi* nucleic acid sequences include SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, or SEQ ID NO:48, and degenerate variants thereof. Specific, non-limiting examples of *P. perniciosus* nucleic acid sequences include SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, or SEQ ID NO:84, and degenerate variants thereof. These polynucleotides include DNA, cDNA, and RNA sequences that encode a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide. It is understood that all polynucleotides encoding a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide are also included herein, as long as they encode a polypeptide with the recognized activity, such as the binding to an antibody that recognizes the polypeptide, the induction of an immune response to the polypeptide, an effect on survival of *Leishmania* when administered to a subject having leishmaniasis, or who undergoes a decrease in a sign or a symptom of *Leishmania* infection.

The polynucleotides of the disclosure include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the *P. ariasi* polypeptide or the *P. perniciosus* polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specific, non-limiting examples of a polynucleotide encoding a *P. ariasi* polypeptide are set forth below:

PRL-P4-A10 (SEQ ID NO:2)

ACTTGTCGATCACTTTTCACTCGCTCCAGACGCATTTTTGCGCTCT
TCAGCCGTGATTAGCACAAAGTGTTTTAGAATTTGGTGAAAAAATAGCA
AGATAAGGATGAAATTAGTGCCATTGTGTATTTTAGTGTGTTTTCTAATC
ATCGCGCAGCAGGTGGCTCAGAATGAAGCATCTCCCGCCAAAAGCCAAG
ACGCCATGTACGGTGATTGGAGTCGTTGGAGCTCCTGTGACGAGACTTG
CCATCAGACGAAGGTGAGATCGTGCCTAGGGGCTGTCTGTGAGCGGAAT
CGACTGATGAAGGAGCGAAAATGTCCAGGATGTGGTACAAAAGTGCGA
ATTGTACAGAAACTTCTTCAGCTCTTCGGCATGGGAGACTCCATAGAGAC
TGACTATGAAGATGACTATGGAGAGCACTGGCTAACTGATGACAGAGTC
ATTAGTTCTAGGAATGATCCTGAAAGTGCAGAAAGTGATGAATTGGGAT
CATTCTTCAGGGATTTCTTCCATTCATTCGATTTTGAGTGGAAAAATCCA
TTTAGCAATCCCCATGAGAACAATGATGTGGACTTGGAGGTGGAGGAAG
ATGAGGAAGTTGAAGAACTTCCCGAAATTAGGACTTCTAATGAGGAGGA
TTCTGTCTCTGGGGCGGATCACGTGTGTGGAGTGACCAAGAATGAGAGA
TCTTCAGGGATGATGGCAAAAACTATCGGCGGGAGGAACTCGAAGAAG
GGTCGATGGCCCTGGCAAGTGGCTCTTTATAACCAGGAATATGAGAATT
TCTTCTGCGGCGGGACTCTTATCTCAAAATACTGGGTCATAACGGCCGCT
CACTGTCTGATATCTGACTTCGGCAGTGACATCACGATCTTCTCCGGCCT
GTACGACACCGGAGATCTCGTGGAGTCGCCCTACAGCATCCACCTGGTG
CGGGATCGAGTGATTCATCCGCGCTACGACGCCGAAACCAATGACAATG
ATATCGCCCTGCTGAGGCTCTACAACGAAGTGAAATTGAGCGATGATGT
GGGTATCGCTTGTCTGCCCAGCTACTCGCAAGCCTCCCCGGGACGCAGTG
AGGTGTGCAAGGTGCTGGGCTGGGGCCAAGGGACACGTCGAACCAAACT
CCAGGAGGCCGACATGCACATCCAACCCGCCAACTCCTGCAAGCGCCAC
TACTACGGCACCGGACAACTCGTCACGCGTCACATGCTGTGCGCCTCCTC
CCGGAACTACGTCAGCGACACGTGTGGCGGTGATTCCGGTGGACCACTG
CTGTGTCGCGACACCAAATCCCCCGCCCGACCCTGGACGCTGTTCGGCAT
CACGAGCTTCGGTGACGATTGCACGGTGAGCGAGAGTCCGGGTGTTTAT
GCGCGCGTCGCCTCCTTCCGGAAGTGGATTGACTCCGTCATCGAGTGCGA

CGGCTCTTGTGACAATTAATAAACTCACAATATTATCAGTGAAAAAATA AATTAGCAAATTTAATGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PRL-P4-A9 (SEQ ID NO:4)

AAAATGAATATCTTATTGAAAGTTGCGATTTTGGTGAGCTTGTGC GAAATTGGGTACTCTTGGAAATATCCCAGGAATGCCGATCAAACTCTCT GGGCTTGGAGATCATGCCAAAAGGGAAACTATGACCCAGAATTAGTGAA GAAATGGATGGCTTTTGAAATCCCAGACGACGAAGTAACTCATTGTTAC ATTAAGTGTGTTTGGACTCATTTGGGAATGTACGATGAAACTAGCCAAA CTATTAGAGCTGATAGAGTCAAGCAACAATTCAAGGCTCGTGGACTATC AGTTCCTGCTGAAATAAGCCATTTAGAGGGATCTACAGGAGGATCCTGT GTAACGATTTACAAAAAAACTAGGGCTTTCCTTGAAACTCAAATGCCGA ATTATCGCATTGCATTCTATGGCACTGTGGAAGAATCAGATAAGTGGTTC GCGAATAATCCCGAAACTAAACCCAAGAGAATTAAGATTTCTGACTTCT GCAAAGGTCGCGAAGCTGGAACGGAAGGAACTTGCAAGCATGCTTGCA GCATGTACTACTACCGCTTAGTCGATGAGGATAATCTTGTGATTCCCTTC AGGAAGTTGCCAGGAATCTTAGATTCCCAACTTGAACAATGCAGGGATC AAGCTAGTTCGGAAACTGGATGCAAAGTTGGTGATACAATCTACAATTG TCTTAACAGAATTAATCCGGAAGGTCTTAAAAAAGCATTGAATACACTC GATGAACAATCATTGACGTTGTATTAGAAAGCAATAAACTTGATTAAGA AAAACAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PRL-P4-C10 (SEQ ID NO:6)

GTCAGTCTTTTGGAAACAAAACATGAAGATCTTTCTGTGCCTTTTT GCTGCAGTTTCCATTCAGGGAGCTTTAGCTTCTCAAATTGAAAGGGAATA CGCGTGGAAAAACATTATTTATGAAGGGATAGATCAAGGATCCTACAAC ATTGAAAACAGCATCCCAACTGCTTTCGCTCACGATGCAGCTAGTAAGA AGATTTTCATCACTATTCCAAGAATAAACCAAGTACCAATAACCCTAACT GAATTTGATAGCATCAAGTATCCGGGAGGTTCTCCTCCTCTTAGCAAATT CCCTGGAAGCGATAACATAATTTCCGTTTATCAACCGGTCATTGACGAAT GTCGTAGACTTTGGATTGTGGACGCTGGACAGGTTGAGTACAAGGGAGA TGAGCAGAAGTATCCCAAGAAAAATCCTGCTATCATAGCTTATGACCTG

ACTAAGGACAATTATCCTGAGATTGATCGATACGAGATACCGATTAATA
TTGCTGGTAATCCATTAGGATTTGGAGGATTTACCGTTGATGTTACCAAT
CCGAAGGAGGGATGTGGTAAAACTTTTATCTACATCACAAACTTCGAAG
ACAACACTCTGATTGTGTACGATCAGGAGAAGAAAGATTCTTGGAAGAT
CAGTCATGGTTCATTTAAACCCGAACATGAGTCGATTCTAATCCATAACG
GGGTTGATCATATTTTAAAACTGGGTATTTTCGGAATCACCCTTGGAGAT
CGGGATTCGGAGGGAAACCGTCCGGCTTACTACTTAGGAGGAAGCAGTA
CGAAGCTCTTTGAAGTCAACACAAAGGCTCTTAAGAAGAAGGAGGGTGA
AATCGAACCAATCACTCTGGGAGATCGTGGACCTCATTCCGAAGCCATT
GCTTTGGCATACGATCCCAAGACCAAAGTGATTTTCTTCACTGAATATAA
CTCTAAGAAGATCTCATGCTGGAACATCAAGAAACCCCTTATTCATGAC
AACATGGATAAGATTTATGCTAGTCCTGAATTTATTTTCGGCACTGATAT
TTCGGTTGATAGTGAATCCAAATTGTGGTTCTTCTCCAACGGACATCCAC
CCATTGAGAATCTGCAGTTGAGCTCTGATAAGCCTCATATTCATCTTATA
AGCGTGGATACGGAAAAGGCAATTCGTGGCACTAAATGTGAAGTGAAG
GCCTAAGTCAAAAATATAACAATTTTACAACAAATTGTAAATTTAACGA
TGATAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PRL-P4-D6 (SEQ ID NO:8)

GTCTTTTTGGAAACAAAGATGAAGATCTTTATGGGCCTAATTGCT
GTGGTTTCCCTTCAGGGAGCTTTAGCTTATCACGTTGAAAGGGAGTACGC
GTGGAAGAACATTACTTTTGAAGGGATAGATCAAGCATCCTACAACATT
GAAAACAGCATCCCAACTGCATTCGTTCACGATGCACTTAGTAAGAAGA
TTATCATCGCTATTCCTAGGCTATATCCTCAGGTGCCAATTACTTTAACTC
AACTTGATACCACCAAGCATCCGGAACGTTCTCCTCCTCTCGAAAAATTC
CCTGGAAGCGATAAATTAACCTCTGTTTATCAACCGATGCTTGACGAATG
TCGTAGACTTTGGATTGTTGACGTTGGACAGGTCGAGTACAAGGGAGAT
GAGCAGAAGTACCCCAAGAAAAATCCTGCTATCATAGCCTATGACCTGA
CTAAGGACAATTATCCAGAGATTGATCGATATGAGATACCGATTAATAT
TGCTGGTAATCAAATAGGATTTGGAGGATTTACCGTTGATGTTACGAATC
CGAAGGAGGGATGTGGTAAAACCTTTATCTACATCACGAACTTCGAAGA
CAACACTCTGATTGTGTACGATCAGGAGAAGAAAGATTCTTGGAAGATC

AGTCATGGTTCATTTAAACCCGAACATGAGTCTAATTTCTCCCACAACGG
TGCTCAGTACAAGTACAAAGCGGGTATTTTCGGAATCACCCTTGGAGAT
CGGGATCCGGAGGGAAATCGTCCGGCTTACTACTTAGGAGGAAGCAGTA
CGAAGCTCTTTGAAGTGAGCACTGAGGCTCTCAAGAAGAAGGGTGCAAA
GTTCGATCCTGTTCGTCTGGGTGATCGTGGGCGTCACACTGAAGCCATTG
CTCTGGTATATGATCCCAAGACTAAAGTTATTTTCTTTGCTGAATCTGAC
TCGAGACAAATCTCATGCTGGAACACCCAGAAGCCACTAAATCATAAGA
ATACTGATGTAATTTATGCGAGTTCCAAATTTATTTTCGGCACCGACATT
CAAATTGACAGTGACTCCCAATTGTGGTTCTTATCCAACGGACAACCCCC
CATTGATAATCTCAAATTGACTTTTGATAAGCCCCATATTCGTCTTATGA
GGGTAGATACGAAAAATTCAATTCGTAGGACTAGATGTGAAGTGAAGCC
CATCAAGAAGCCATAAGACAATCTATTAAAAATGTAACAATTTCCCCAA
AAAAAGAAATTGTAAATTTTACGATGATAATAAAAAAATTTTATGCTTGT
GAAAAAAAAAAAAAAAAAAAAA

PRL-P4-D7(SEQ ID NO:10)

ATTCAGTCATAAACCTGGGGTAATGTTTAAGGAAATTATCGTAGT
GGCTCTAGCCGTGATCGTGGCACAATGTGCTCCTCCTGCAATTCCAATTG
CAAAACAGGGAAACGATTTCCCTGTCCCAATTGTTGATGAAAAGGAAAC
GGATGATTTCTTTGACGATCGATTCTATCCGGACATAGATGATGAGCGTG
TAGGTGCTAGGGCTCCGGTGGGTGGCAAACAGACATCTAATAGAGGAAC
CAGTTCTCAGAGTGATAAGGTTCCTCGTCCTCAAGGGTCCAATAGAGGG
CCTAGCTCTCAGACTACTGACAAGGTTCCCCGTCCTCAATGGCCCAGTAG
AGGAACCAATTCTCAGAATGACAAGGTTCCTCGTCCTCAAGGGTCTAGT
GGACAAACTCCACCTAGAACGCCTGGAAAGGTTGAACAAAGTGGAAGG
ACCAACACAAAGGACCAAATACCTCGTCCACTGACTAACAGAAACCCAA
CCAAGAACCCAACTGAACAGGCTAGAAGACCAGGAAACAGGGAGCTAC
TCATTAGGGATAAAACCCCAGGGAGTCAAGGTGGAAAACAGGGAACAG
GCAATAGGCAGAAACTGTCGAGTTATAAAGACGCTCAGCCGAAGTTGAT
TTTCAAATCGAGTCAATTCAATACTGATGGCCAAAATCCATATTTAACGA
GGTTGTTCAAGACGAAGAAAGTCGAAGAAGTTATAGCTAAAGGAAGTCC
CACTGATGAATATGTCCTGGAGCTTTTGGATGGAAAGCCAGATAATCTG

AGCTTGGTCATCAGAACAAATGGCAAGACGAGCCAAGCGGTTCTCAGGA
ATCCCACTCGCAACAGAATTGTGGGCCGTATCAAGTCGTACAACCCCGG
CCCGAGGCGAATGTCCTATTAATTTTTTTTCTCTTTTTCTCCTAAATAC
AACTCCCACAATAAAATTTCAGTTGTACGCAGCAAAAAAAAAAAAAAA
AAAAAAAAAAAAA

PRL-P4-E5 (SEQ ID NO:12)

TAACGCTCAAGCTTTGTCTTCAATATGGCTTCCATCAAGCTCAGTA
CTTGCTCTTTCGTTTTGCTAAACCTCATTCTACCAACAATCTCTATGAAAG
TTATCAGTTTCGACGATAGAGATGAGTATCTACTTGGTAAACCTGCAAAT
TCTGACGATGAACTTCTCTATTCAACCTTTGACTTCCAGAGAGATCCCTG
TTCTAAGTCTTACGTGAAGTGCACCAACAACAACACCCACTTTATTCTGG
ATTTCGTTGATCCGAAGAAGAGATGCATCTCTTCAATTCACGTTTTCTCC
TACCCCGATAGACCTCCCAGCTTTGAGGAGAAGAGGATTCCCTCGAAGA
GTGCAATTTACTGCCAAAAGGGCGGCATTGGGAAGAGTCACTGTTTGCT
GGTGTTCAGGAAGAAGGAACCTCGAGAGGACGCACTGGTTGATATCCGG
GGAATCCCCGCTGATCAAACATGCTCCCTCAAGGAGCGCTACACATCGG
GAGATCCTAAGAAAACCGATGCTTACGGAATGGCCTATCAGTTTGATAG
AAAAGATGATTGGCACATTCAAAGAACAGGTATCAAGACATGGAAAAG
ATCAGGAAACGAGATCTTCTACCGTAAGAATGGTTTAATGAACCATCAA
ATAAGGTACTTGAGCAAGTTCGACAAATACACGGTTACCAGAGAATTGG
TGGTGAAGAACAACGCTAAGAAATTCACCTTGGAATTTTCAAACTTCCGT
CAATACCGAATCAGTTTCTTGGACATCTACTGGTTCCAGGAGTCTCAGAG
GAATAAACCCAGATTACCTTATATTTACTACAACGGTCATTGCTTGCCTA
GCAACAAGACATGCCAGTTGGTCTTCGACACTGATGAGCCTATTACTTAT
GCTTTTGTGAAAGTGTTTAGTAATCCGGATCACAATGAACCACGACTAA
GACATGAAGATCTAGGACGAGGGTAAGAATGGACTAGTCCGGGGTTGA
AAAATCGCCTAAAATATGGGGAATCTATTATTGAAAAAAAAAAAAAAA
AAAAAAAAAAAAAA

PRL-P4-F3 (SEQ ID NO:14)

ATGTATCAAACATCACGGATATGGTGATTTATCTCACGCAGAATATCAGTAGAGCTCTTCTCACGCTTCTACCAAATCCTGAAGATGTCCGATCAGCAGCGGATGTCCTAGAAAGTTTTACAGACGACCTCAAGTCTTTCTACCCACCTCCTGATGATGTGAATGAAGAGGTATCAGAGACAGAGTCAAGAACTAAGAGATCATTGATTGAGCAACTCAAAGAGTCGCAACCTCTAAAACAAATCAGAGAAACAGTTGCTGAGACAACCAAGTACCTAAAGGGATTCTTAAAAACGAAACCTTCTGGAAATCAAACGGAGAGTTCTAACTCAACAAGTACGAAGACTCAGTCAAGAAAGAGACGTGGATTAACTGATTTTATACCAGTGAATTCTCTAAAGGATGCAATTTCACAAGCAACTTCAGGTGCCATGAAAGCGTTCAAACCTTCAAGTGAAAATAAAACAAGTTCAAATCCTCTAGATTTCCTCGCAAGCCTCTCAGATATTTCCAGAGATCTTGTACAAAATTCAATTAAGGAAGTCTCTGGCAATTTAGTTTCAAGCGTTGCTTTATACCAAGTCAACTCAAAGTTAGATGCCATTAAACAATCCATTGGTATCATAAATCAAGAAATTGATAGGACCAAAAAAGTTCAGCAATACGTCATGAATGCTCTTCAACAAGCCAGCAATATTACTAACTCAATTGGAGAGCAACTCAAGTCCAACAACTGTTTCGCACAATTTATAAACCCATTCAAACTTTTCGAAGAAGTAATAACTTGTGTGAAAAATAAAATCGAAAATGGATTGAAAATTGCGGAAGAGACATTTAAAAATTTAAATCAGGCTTTAAGTGTGCCCTCAGATATTGTAAGTGAAGTGTCCAAATGTTCCCAAAACCAGAACTTGAATCCCTTGACGAAACTTCTGTGCTACTTGAGGGTACCCCTGCAATTGGATGAGGAGAAACTGCTGCTTCCTATTGAATTTGCGAGGCGAATTAGAGAAATAACCAACTATTTTGCCACCATGAGAATGGACCTCATTCAATGTGGCATAGCAACTATTCAATCAATCGGAGACAAGGTTGAGAATTGTGCAATAGAAGCAATATTGGCTGTAAAGGACACTTTGAAGGGATAAAGTCCGTATTTTATGCTGTCCAATTGGGCTAACCCAATCATTGATATACCGAATTGTGTATGTATATTGAGAAAATGAATAAATGCTTCAAAATGAAAAAAAAAAAAAAAAAAAA

PRL-P4-G12 (SEQ ID NO:16)

ACATACGATTCCTAACCAACCATGAAGCAGTTCCCAGTGATCCTTTTGACCTTAGGCCTTTTGGTCGTGAAATGCCGATCAGAACGGCCGGAATGGAAATGTGAAAGAGACTTCAAGAAAATCGACCAAAATTGCTTTCGTCC

TTGTACATTTGCAATTTACCACTTTGTTGATAACAAGTTCAGGATTGCCA GGAAGAATATTGAAAACTACAAGAAGTTCTTAATTGACTATAACACCGT GAAGCCCGAAGTTAATGATTTGGAAAAACACCTGTTAGATTGTTGGAAT ACAATCAAATCCATTGAAGCATCATCCAGGACGGAAAAATGTGAACAAG TTAACAACTTTGAACGATGTGTTATTGACAAGAACATTCTTAATTATCCT GTTTACTTCAATGCTTTGAAGAAAATAAATAAGAATACAAATGTTTAATT AAATAAAGATGTGAAATATTGCAGTGCACAAATATAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PRL-P4-G7 (SEQ ID NO:18)

AGAAGTTATTTTACACCTGTGCAATGATAAACCCAATAGTGCTGA GATTTACTTTTCTCTTGGTGATTTTGTTGCCTGGCAAATGTAAAAGTGCCC CAAAGTCTTGCACCATTAATCTTCCCACCAGCATTCCCAAGAAAGGTGA ACCGATTTACCTCAACAGTAATGGATCAGTTTTCCGACCTATTGGAGGTT TAACTCAACTCAACATTGGGGACTCCCTCTCCATCTACTGTCCACCACTG AAGAAGCTCAAGAGTGTTCCTTGCAGTCGAAAATTCTCCCTTGAGAGCT ACTCTTGCAACAACAGCTCTCAGAGTGAACTCGTGCAGACGGAGGAGGA GTGCGGACAAGAGGGGAAATGGTACAACATTGGCTTTCCATTGCCCACA AATGCCTTCCACACAATCTACAGAACTTGCTTCAATAAGCAGAAACTAA CACCAATTTACTCTTATCACGTCATCAATGGAAAGGCCGTTGGATATCAC GTGAAGCAGCCGCGAGGAAACTTCCGACCAGGAAAAGGTGTCTACAGG AAAATCAACATCAATGAGCTGTACAAGACGCACATTTCGCGCTTCAAGA AAGTCTTCGGTGACAAACAGACATTCTTCCGGAAGCCACTGCACTACCT GGCTCGCGGACATCTCTCCCCTGAAGTGGACTTTGTCTTCGGCACCGAAC AACACGCCACTGAGTTCTACATCAACACCGCCCCCCAGTATCAGTCCATC AACCAGGGAAATTGGCTGCGAGTTGAAAAACACGTGCGCGGTCTGGCCA AGGCGCTCCAGGACAATCTCCTCGTCGTCACTGGCATTTTGGACATCCTA AAGTTCTCAAACAAACGAGCCGACACAGAAATCTACTTGGGCGACGGAA TAATTCCTGTTCCGCAAATATTCTGGAAGGCAATCTTCCACCTCAGAACA TCTTCCGCCATTGTCTTTGTCACCTCCAACAACCCTCACGAGACGACCTT CAACAATATCTGCAAGGACGCGTGTGAAATGGCAGGATTCGGAGACAAA CAACATGGAAATCAAAATTTTTCCAACTACTCCTTGGGATTCACCATCTG

TTGCGAACTACAGGACTTCATTGGGAACTCGAAAGTTGTTCTTCCAAAGG
ATATTCAAGTCAAAAACCACCGCAAACTTCTTCAGTTGCCAAAACCGAA
GCAATAAACTTTAATTTTGGTCTTGCAAAGTGTGAGTATATTTTAAATAA
ACAGCAAATCAAAAAAAAAAAAAAAAAAAAAAAA

PRL-P6-E11(SEQ ID NO:20)

AGTCTCTCCCAGGGTTTTATTGTGGAAAATGAACGCTTTATTGCTT
TGTGTTTTGTTGAGTTTAAGTGGAATAGGGTACTCTTGGAAATACCCTAG
GAATGCCGATCAAACTCTCTGGGCTTACAGAACGTGCCAAAGAGAAGGG
AAAGATCCGGCATTAGTATCCAAGTGGATGAATTGGGTGTTACCAGATG
ATCCGGAAACTCACTGCTACGTTAAGTGCGTTTGGACCAATTTAGGATCC
TACGATGATAACACCGGTTCCATTATGATTAACACAGTGGCTACACAATT
TATAACACGCGGCATGAAAGTCCCAGCCGAAGTAAATAATTTAAGTGGG
TCGACAAGTGGATCTTGTTCAGATATTTACAAGAAAACCATTGGGTTCTT
CAAAAGTCAAAAGGCGAACATACAGAAAGCGTATTACGGAACTAAGGA
AGAGTCAGATAACTGGTATTCGAAACATCCAAATGTAAAGCCGAAAGGA
ACGAAGATTTCTGACTTCTGCAAAGGTCGCGAAGGTGGAACGGAAGGAA
CTTACAAGCATGCTTGCAGCATGTACTACTACCGCTTAGTCGATGAGGAT
AATCTTGTGATTCCGTTCAGGAAGTTGAAAATTCCGGGAATTCCAGGACC
CAAAATAGATGAGTGTAGGAGGAAGGCTAGCTCGAAAACTGGATGCAA
AGTTGCCGATGCACTATACAAATGTCTTAAGGCTATAAACGGTAAAAGT
TTTGAAAATGCTTTAAAGAAGTTGGACGAAGAATCATCCAGAACTTATT
AAAATAAAAGAAACTTGAGTTGCTAAAAAAAAAAAAAAAAAAAAAAAA
AAAAA

PRM-P3-A6 (SEQ ID NO:22)

ATATCTAGAGGAAAATATTAAGTGAAAAGTGAAATGATTAGGATT
CTGTTTCCTCTCTTTATTCTTAGTCTTGGAATTTATCAAGTAACTTGCCTT
ATGTGCCACTCATGTACTCTTGATGGGGAGCTTGAGTCATGTGAAGATTC
TATCAATGAGACTTATGTAGTTAAGATTGAGGAAAAGGAGTGCAAACCT
GCGCAATCTTGCGGAAAAGTCTCATTTACTGCGAATGGAACAGTTCGAA
TCGGAAGAGGATGTATTCGCTCAAGCAGTAGTTGGAAAATCGATTGCAG

AATACTTGCAAAGGAAGTTAGAGATGAAGGCATTGCGGTAACACACTGT
TCCTTATGCGACACGGACTTGTGCAATGAATAAATAAAATTGTGAAGAA
AAAAGTATTGTAACTGTTACTGGAAAAACAATTTCAGAAATATCCACAA
TAAAAAGAGAGCATTTCGCTGTAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAA

PRM-P3-F11 (SEQ ID NO:24)

TCCAGTTAATATTCCGACATGTTGCAAATTAAACACTTCTTGTTCT
TTGTGGTGTTGTTCGTTGTCGCTCACTCCAATGATTATTGTGAGCCGAAA
TTGTGCAAATTTAACAACCAAGTGAAGACTCACATTGGATGCAAGAATG
ATGGAAAGTTCGTGGAAAGCACTTGCCCAAAACCAAATGATGCTCAAAT
GATTGATATGACTGAACAGAGGAAGAATCTCTTTCTCAAGATTCACAAT
CGCCTTCGCGATAGGCTCGCTCGTGGTTCTGTGTCTAATTTCAAGTCAGC
CGCCAAGATGCCAATGCTGAAATGGGACAATGAATTGGCCAGGTTGGCA
GAATACAATGTGAGAACGTGCAAATTTGCTCACGATCAGTGTCGCTCAA
CCAAGGCTTGTCCTTATGCTGGCCAGAACTTGGGCCAAATGTTGTCTTCT
CCAGATTTCTTGGACCCCAACTATGTCATCAAGAATATCACTAGGGAGTG
GTTCTTGGAGTATAAGTGGGCAAATCAAGGACATACTGATAAATATATG
ACAGGATCTGGTAAGAATGGCAAAGCAATTGGTCACTTTACTGCCTTCAT
CCATGAGAAAAGCGACAAGGTTGGATGCGCTGTTGCTAAATTAACCAAC
CAGCAGTACAACATGAAGCAGTACCTCGTGGCCTGCAACTACTGCTACA
CGAATATGCTAAAGGAAGGGATCTACACGACAGGAAAGCCTTGTTCTCA
GTGCCAGGGAAAGAAGTGTGATTCCGTCTACAAGAACTTATGCGATGCG
AGTGAGAAAGTCGATCCCATCCCAGACATCTTTAAGCAATCGAGACAAC
AGAGGAGCAGGAAATAATTCTCTGCTTTCCCATTTGGTATAAAATGTTAA
ATTTATTGTTTTCCCATCTATTGGGTGAATTGGCGAAAAAGGTGAAGATG
AAAAAAGGTATAAGAAAATAAGAGATAAACAGAAACTGAGATATCTGA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAACCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PRM-P5-D6 (SEQ ID NO:26)

TCAGTTTCACTTTGACCATCGATGGTGCAATTACTTCAATTCAATT
TACGAAATCACTTTGATTGAGAAACGATGATCGTGAAGAGTTTCCTTGG
GGTGTTTCTTGTGATCTTGCTCGTGTCCGTGACAGAACAGGATCGTGGAG
TAGACGGACACAGGAGGACTCAAGATGACCATGATTACAGCGAATTGGC
GGAATATGACGACGAAGATCCTCATCAAGAGGTAATTGACGGTGATGAG
GAGGAACATGAGTTGTCCGGAGGACGTCGACTATCCCACGAAGACGAAG
ACGACGACGACAGACACTATGGCCATCGTGGAGAGGATCGAGAGAATTC
TCGAGGCAGAAATGGTGGATCTCGTAATCGTGGTAGTGAGGAACAATCA
TACGATCCCTACAGCCACGAGAGAGCTCCTACCTACTCAGAATCCAGTG
AATACGACCACAGCGGTGACTACGACAATTCCAACTACCAGCAACATTC
CTCCACTCCCTCCTCCTACAGCAACATCGATCACTATCTCCATCTCATCC
AATTGCACAGCGTCCCCAGTGATTTAGCCCAGTACGCCGATTCCTACCTT
CAACACTCCAAGAACTCCATCAGATACTACGCTTCGCATGCCAAAGACT
TTGAGAAGATTCGACCCTGTCTGGAGAGCGTCGTGAAGTACTCCAATCTC
CTCAATGACGATCTTGCCAAGGAGTACATCAGATGCCAACGAAAGTGTT
ACCTTGAACGTCTCAATAGCTACACATCGGCTATCTCTCAGTACACAGTC
ACCACAAATGCCTGCATAAACAACCGATTGCATTAAAGCTGAGGATTAT
CTTGTGAAATATTTATTTGAATCGATCAGTGAAAATAAATTTCCAATAGC
AAAAAAAAAAAAACAAAAAAAAAAAAAAA

PRM-P5-E9 (SEQ ID NO:28)

AAAGTATTCAGTTGTGAGAAATCTTTCCAAATACACATCATGATT
ATCAAATTGTGCGCTATTGCTGTTGCTTGTCTCCTCACTGGAGATGGAGA
AGCAGCTCCCAGAGCAACAAGATTCATCCCTTTCGCTGTTATCTCCGACT
TGGACAAGAAGTCCATTAAATCCGATCAGAAGAGTTTCACCAGCATCGT
GAGATATGGCGAATTGAAGGACAATGGAGAGAGATATACGTTATCCATC
AAGAGTGAAAATCTTCACTACTTCACGCGATACGCTTACAATGGACGCG
GAGCCGAATTATCTGAATTGTTGTACTTCAACAACAAACTCTACACCATT
GATGACAAAACAGGAATTATCTTTGAGGTGAAACATGGTGGGGATCTCA
TTCCATGGGTGATCCTGTCGAATGGCGATGGAAATCAAAAGAATGGCTT
TAAAGCCGAATGGGCGACAGTTAAGGGTGACAAGTTGATTGTCGGATCA

ACAGGAATCCCCTGGTTTGAGGAGAAAACCCAGTCTCTTAACACCTACA GCCTTTGGGTGAAAGAGATCAGCAAGGAAGGCGAAGTCACCAACATCA ATTGGAAGAGTCAATACAGCAAAGTGAAGAATGCAATGGGAATTCCTTC CTCTGTGGGATTCGTCTGGCATGAGGCTGTAAATTGGTCACCGAGGAAG AATCTATGGGTCTTCATGCCCAGAAAATGTACAACTGAATATTTCACCAG TCAAGTGGAAGAGAAAACTGGATGCAATCAGATTATCACGGCTAATGAA GATTTCACTCAAGTGAAAGCAATTAGGATCGATGGACCTGTTCAGGATC AAGCTGCTGGATTCTCCTCCTTTAAGTTCATCCCAGGCACTCAAAACAAT GATATCTTCGCACTGAAGACTATCGAGAGGAACGGCCAAACAGCCACTT ACGGGACAGTAATTAACATCGAAGGGAAGACTTTGTTGAACGAAAAACG AATTCTCGATGATAAATACGAAGGAGTTGCATTTTTCAAGAATCCCGAA GGCATTATATAAAATAATAATGATGGAGTGAAAAACAAATTGAAATAAA ATGCTAAAGCTCATAAAATTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PRM-P5-F12 (SEQ ID NO:30)

ATCAATTGTTATTGAAATAATCTTCAAGATGCATTTCAAGATTATC TTCTGCTCCCTCTTCATTGTCCTGCTGGGACATATGGCGTTTGCTGAATCT TCTGAGTCATCATCTTCAGAGTCGTCGTCTTCAGAAACATCTGAAGAGTC ATCTGAAGAAGTTGTTCCATCCCCTTCTCCCTCACCTAAGCATCGGCCGC ATTTTGGTCCCCATCACCCACATGGAGGCCGACCTAAGCCTCCCCATCCG CCGCCACCGAAACCTGAGCCGGAGCCAGATAATGGCTCAGATGGTGGCA ATCAGGATAATTCAAATGGTCAGGATAACTCTAATGGAAACTCTCAGAA TGATGAACAGGATAACTCTCAATCGGGATCCGCTAAGCGATTCAGACAA CCTGCAGTGAATATTGTTAATCTTGTGATTCCTTTTTCTACGATTTAACTT TCCTTTTGTCTACTTTAATCACTTTAATGCACGTAATAATAAAAAATACTT TCAGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PRM-P5-F2 (SEQ ID NO:32)

TTTCTCAATTTGTGTGTGATTGCTCTAGACGTGGCCGGTGAATTTT CCCAAAATGTTTTCCAAAATCTTCTCTTTGGCTATCCTCGCTCTCGCCTTG TCCACTGTGTCCAGTGAGACTTGCAGTAATCCTCAAGTGAAGGGCGCTTC

CTCCTACACAACCACGGATGCAACGATAGTCAGCCAAATTGCCTTCATC
ACTGAATTCTCACTGGAGTGCTCGAATCCCGGAGCTGAGAAGGTCTCCCT
GTTCGCCGAAGTTGACGGACGGATCACTCCAGTTGCGGTAATTGGAGAT
ACTAAATATCAGGTGAGCTGGAATGAGGAGGTCAAGAAGGCTCGCAGTG
GAGATTACAATGTAAGACTGTACGACGAGGAGGGATACGGAGCTGTGCG
CAAAGCCCAGAGATCAGGAGAGGAGAACAATGCGAAGCCACTGGCTAC
AGTTGTTGTTCGCCATTCCGGATCCTACACTGGCCCTTGGTTCAATTCTG
AAATCTTAGCCTCCGGTCTCATCGCCGTCGTAGCATATTTTGCTTTCGCC
ACCAGAAGCAAAATCCTGTCGTAGAGACGCATCAATAATTTCACAAAAA
TGTAGCCAGAAGGCTGTTCTTGGCACTCAGACTGTTTCTGTGAAATACAA
CAACATATCAAAAAAAAAAAAAAAAAAAAAAAAA

PRM-P5-G11 (SEQ ID NO:34)

GTTTCTTATACATCACTTTGAAGCAGCAATGAGTAACTTGCTAACT
ATCTTTGGGGCAATTTGTTTCTTGGGCGTTGCCAACTCTCTGCAATTCCCT
CGGAACCCAGACCAAACCAGATGGGCAGAAAAGACATGTTTAAAAGAA
TCTTGGGCACCACCTAATCTGATAAACAAGTGGAAGCAATTGGAATTTC
CCAGTACCAATCTCACCTACTGCTACGTGAAGTGCTTTGTTATGTATTTA
GGAGTCTACAACGAGACGACCAAGAAATTCAACGTAGACGGTATCAGAT
CCCAATTTACAAGTCAAGGACTTCGTCCACCTAACGGTCTAGAGAGCCT
ACAAAAGACATCTAAAGGAACCTGCAAGGATGTCTTCCGAATGTCCGCT
GGCCTAATCAAGAAGTACAAATTGGAATTCGTAAAAGCTTTCCATGGAG
ATTCTGCCGAAGCTGCGAAGTGGTACATCGAACATAAAGGAAATGTTAA
GGCAAAGTATCAGAAAGCTTCGGAATTCTGCAAAACTCAGAAGGATGAG
TGTAGGCTGCATTGTCGTTTCTACTACTACCGCTTAGTTGACGAAGACTT
CCAAATATTCAATAGAAAATTCAAGATCTACGGCATTTCGGACTCACAG
CTACGGCAGTGTAGGAGTAAAGCCAGTCAAGCTAAGGGTTGCAAGGTTG
CCAAAGTCCTTAAAAATTGCCTCGACAAGATTGATTCTGAGAAAGTGAA
AACCGCTCTTAAGACTTTGGATGAGATATCAGCAAATTACGTTTAACAGT
AATCTCCAAGTTAGCCCCATCAGCCTAATTTAGCGCCACCTTTAAATCAA
CCCCCAGCTAATTTCTCGAACGTTAGAAAAAGGTGTTTAACTTACGGGTG

ATTGAGTGTAAGTAATTTAGCGGCTGTGGAGATGAAATGACTATTAAAT
CGTGCACAATGGGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PRM-P5-H4 (SEQ ID NO:36)

ACAATTCATATTTCCTTTAGTGAAGTTGTTGAAAATCAAGCAAGA
TGTACTTTACCCATACCCTCAATTTTCTTCTTCTTGTAATTCTATTAATAA
TGGCTGGTTTTTCCCAGGCAAATCCCGAGAAAAGGCCCTGCACAAACTG
TGAGCGTCCCAAGTTATCGGCTAAAACTCCTTTGTAACCCTTTTAAATCA
TATAATCGGTGATTAAAGATTTACCAGCAGAGCTACCGCAATGTGAAAT
CGAAAAATTATACCTACCTACAGAAAAACTAAAATGTAATAAGAATTAG
AAAAAATAAAAATGATCCAAGAACAAAAAAAAAAAAAAAAAAAAAAA
AAAAAA

PRS-P1-B11 (SEQ ID NO:38)

ATCGCGATTCTGTTGCAACGTCACAGAGTACTTCCTTCTTTTCCTT
TCGGTTTCCTATCATTTCATTTGTTATCTCGCACCCAAATGACGTGGGTG
ATTCTTTGTGTCGCCCTCCTGGTTGCTTCCGTTGTCGCGGAGGGCGGAAT
CGATGCGGAGGGGAATCGCACGAAAATCGAGAAGATAACCGCGGGTGC
AGGAAGTGATGGCAAGGTGGTCTACACAGAGGGTGGAAGCTTCCCGGA
GAAGCTAGAGAAGGAGCAGAAGAGCGTGAAGAAGGAGCTTGGAGAATT
GCCAAAGCCCACAAATGCCACATTTTCACCTCCCGTGAAGGTGGAGAAT
AAGACGGAGGAGGTGAGGAATGCTACACTGCCGGTGAATGCCACAACT
GAGGCCCCTAAGGTGGTCAATACGACAGCCAGCACCACCACGGTGAAGC
TAACATCCACCAGCACCACAACAACTACTCCCAAGCCCAAGAAGCCCAG
CCTCACGATTAGCGTGGAGGACGATCCGAGCCTCCTGGAGGTGCCAGTC
AAGGTGCAGCATCCACAGACCGGAGGACGACTGGATGTGGAGGAGCCT
GTGGCTCAGCTGTCGCATGAGAACATCCTGGAGATGCCCGTGAATCACC
GGGACTACATTGTTCCCATTGTGGTGCTTATCTTTGCCATTCCCATGATCC
TGGGACTCGCCACTGTTGTCATCCGACGTTTCAGGGACTACCGGCTCACT
CGCCACTACCGCCGGATGGACTACCTCGTGGATGGAATGTATAATGAGT
AGTTTCCGGCTCGCACTAACCGCCCAAGCAATAATCTAATTAATGCTTAA

TCGTTTTATACTATGTAAATAAATGTACATTTTAATAATAAAAAAAAAA
AAAAAAAAAAAAAAAAAA

PRS-P1-B4 (SEQ ID NO:40)

CGTCAGTTTGTTGAAAGTTGGGAAAATGAAGAAAATTCTGCTATT
CAGTGTTATATTCGTTGCTTTGTTGATCACTGCCGAAGCCATTCCGGGAA
AACGGGCAAGACCGAAAGCTCCCGCGGTCACTAAAGGTCGGGATGTTCC
AAAACCAAGACCTGGTCAAGGAGGACAAGTGCCAGTTGAACCAGATTTT
CCTATGGAAAACTTAAGAAGTAGAATTTAGTAGATCTTCAGCTTTCTCGG
CCCCTTTAATAAAATTCGTCTACTGATAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAA

PRS-P1-E7 (SEQ ID NO:42)

ATATATCTATCGATTTCTCGTGTTTTGATTTGCTTAGGTGGCCCCA
TTTTTCCAAGAAAATTCCTGAAATGGCAGTTAAAAATCTTCACAAATTCC
TCCTGGTCGTGGGATTCGTGTCCCTGATCCATGCGGCTTATTCGGCAGCA
CAGCACAGAACGTACCTGAGAATCACGGAGCAGGAGTTTAATTCTCTCC
CATTTGACATTGTGCTCCAAGCTGTGGTGAGTCTGATCATTCTGGTGTAC
AGCATTCTGCAGGTTGTTGGGGAGTTCCGGGAGATTCGAGCAGCTGTGG
ACTTGCAAGCGAAATCATGGGAGACTTTGGGTAACATCCCCTCCTTCTAC
ATGTTCAATCACCGTGGGAAGAGCCTATCCGGCCAGTATGAGGATAACA
TTGACACGAGTGCCGATTGAATGCCCGGAAGAAGCCTTCCCGTAAATCT
ATTTGAATGTAAGGAATCCGATTAATTGAATTAACACCAAAGGAGAGCT
GAGGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PRS-P1-G9 (SEQ ID NO:44)

CACGAATTAGAAAACGGTCCCAGTGATTCTCTCGGTGGCTGATTT
ATAAGAGAATGTGAAGAGTTGAGGATGATGTCTCGCTGGAGCAAAAGTG
TGAAATTTGTGTGCCTCCTCCTGTGTGGCGGATTCACGTTTCTCACAACA
TCAGCACGTGCCAAACCCACACTGACCTTTCAGCTACCGCCCGCACTCAC
GAACCTACCCCCCTTCGTGGGCATCTCACGATTCGTCGAACGCAAAATGC
AGAATGAGCAGATGAAGACCTACACTGGCGTTCGGCAGACGAATGAGTC

TCTCGTGATGATCTACCACCATGATCTGACGATCGCCATCGTGGAATTGG
GACCAGAGAAGAGTCTCTTGGGTTGTGAATTGATAGAAATTAACAACGA
TGACGAAGGCGCCAAAGTGCTGAAAGAACTGGCCACGGTGAATATACCA
CTGGAGATCGACTTCCGGGAGATGGTGAAGCTCATGAAGCAGTGCGAGA
AGATCGATTACATACGGAAAGTGAAACGCCAAGGAGCACCAGAGAGTG
ACCAGACGACAAATCGTCAACACCAGACGGGCTACTTCACGGGCGCCAC
TGCCGGCCTGAGTATCCTCAGTGGCATCCTTCCCGGCACCAAGTGGTGTG
GCACAGGAGACATCGCCAGGACATATCACGATCTCGGCACAGAGGCTAC
CATGGACATGTGCTGTCGCACTCACGATCTCTGTCCAGTGAAAGTGCGCT
CATATCAGCAACGCTACAATCTCACCAATAAGTCAATCTACACAAAATC
TCACTGTAAATGTGATGACATGCTGTTCAATTGCCTCAAGAGGACCAAC
ACGTCAGCCTCGCAATTCATGGGGACCATCTACTTCAACGTGGTCCAAGT
GCCATGTGTTCTGGACACAGACAGAGGCTACAGATTCAGAAAAGCGAGA
ACCTTCTCCTGATCATCGCAATGCAACGAAATCTGAGGATATTTTATTTT
TGGGGACTTTTTTTTGCGTGTAAAGACCATTTCTGTGATTTTCAGCTGAG
GTGCTCTTTCAAATGAATTATTTATATGTTACAAAAAAAAAAAAAAAAAA
AAAAA

PRS-P2-C8 (SEQ ID NO:46)

AACATATCTGAACCAGCCATGAAGTTGTTACCTATAATTCTGTTG
GCGTTGACAGTCTTGATCGTGACTTGTCAAGCTGAACATCCCGGTACTAA
GTGTAGAAGAGAATTCGCAATAGAAGAAGAATGTATCAATCATTGTGAA
TACAAACACTTTGGCTTCACAGATGACCAATTCCGGATTAAAAAGCATC
ATAGAGAAAATTTCAAAAACGCTATGAGTCATTACGGTGCAATCAGAAA
GGATCAAGAAGGTGAACTGGATAAGCTTTTGAATAGATGTGCCAAGAAA
GCCAAAGAGTCTCCTGCTACATCGAAAAGAGACAAATGTTACAGAATTA
TTAACTACTACCGTTGTGTTGTTGTAGATAATAATCTGATCAATTATTCTG
TTTACGTCAAAGCTGTTACCAAGATTAATGATTCAATCAATGTATAAAAA
TCAAATATTACTTTGAAATAAAAGAAGAAACAATGTTGTATGCAAGGCC
AAAAAAAAAAAAAAAAAAAAAAAAAA

PRS-P2-G8 (SEQ ID NO:48)

ATAGAAATCGAATCATGAAGGAGCTTGTTGTATTTTTGACACTGA
TAGTTTTGGTCGTGATTTGTCACGCAGAACGACCTTCACAAAAGTGTAGG
AGGGAACTGAAGACAGAGGAAGAGTGTATACTGCATTGTGAGTACAAA
CATTATCGCTTTACTGATGACCAGTTTCGACTTAACGCAGATCAAAGAGG
AGACTTTAGGAATATCATGAGGAGGTACGGCGCAATTAGGGTGGATCAG
GAAAGTCAATTGGATAAGCATTTGAAAAAATGTGCCAACAAAGTTGCTA
AGACTCCGGCAACATCGAGGAAGGATAAGTGTAGGAAAATTTCTCGGTA
CTATCACTGTGCTGTGGATAATAAACTTTTCAAATATAATGATTATGCCA
ATGCCATAATTAAATATGATAAGACAATAAATGTTTAAAGATGAATGTA
TCGCTCAAATAAAGAAGCAAAGCTAACCATATTCAAATCAAAAAAAAAA
AAAAAAAAAAAAAAAAAAA

Specific, non-limiting examples of a polynucleotide encoding a *P. perniciosus* polypeptide are set forth below:

PERL-P7-G8 (SEQ ID NO:50)

TATATATAACTTTTGAAATGTTCAGTCAGTCTTTTGGAAGCAAAGATGAA
GATCTTTCTGTGCCTAATTGCTGTGGTTTTCCTTCAGGGAGTTGTAGGCTT
TCACGTTGAACGAGAATATGCGTGGAAAAACATTAGTTACGAAGGAGTA
GATCCAGCATTATTCAATATTGATAATATCATTCCGACTGGTTTCGTTCA
CGATGCAATTAATAAGAAGATTTTCATTGCTGTTCCAAGGAGATCTCCTC
AAATACCATTTACCCTAACTGAACTTGATACCACCAAGCATCCGGAACG
TTCTCCTCCTCTAAGCAAATTTCCTGGTAGCGATAAATTAATCAACGTTT
ATCAACCGGTCATTGACGAATGTCGCCGACTTTGGATTGCGGACGTCGG
ACGGGTTGACTACAAGGGGGATGAGCAGAAGTATCCAAACCAAAATGCT
GTTCTCATAGCTTATGACCTGACGAAGGAAAATTACCCAGAGATTCATC
GATACGAGATACCAAGTAAAATTGCTGGTTCAAATACAATTCCATTTGG
AGGATTTGCCGTTGATGTTACGAATCCGAAGGAGGGATGCGGCAAAACC
TTTGTCTACATCACGAACTTCGAAGACAACACTCTGATTGTGTACGATCA
GGAGAAGAAAGATTCTTGGAAGATCAGTCATGGGTCATTCAAACCAGAG
CATGACTCGACTCTCTCCCATGACGGTAAACAGTACAAGTATAGAGTGG
GTTTATTCGGAATTACTCTTGGAGATCGGGATCCGGAAGGAAATCGTCC

GGCTTACTACATAGCCGGAAGCAGTACGAAGCTCTTTGAGATCAGCACTAAGATTTTGAAGGAGAAGGGTGCCAAATTTGATCCTGTTAATTTGGGAAATCGTGGTCCCCACACTGAAGCTGTTGCCCTGGTATATGATCCCAAGACAAAAGTTATCTTCTTTGCTGAATCTGACTCCAGGCAGGTCTCTTGCTGGAATACCCAGAAGCCACTGAATCATAAGAACACTGATGTGATTTTTGCCAGTGCCAAATTTATTTACGGCTCCGATATTTCAGTTGATAGTGAATCTCAATTGTGGTTCTTATCCACGGGACATCCACCCATTCCTAATCTCAAGTTGACCTTTGATAAACCCCATATTCGTCTTATGAGGGTGGATACGGCTAAAGCAATTCGTAGAACTAGATGCGAAGTGAAGCCCCGCAAGCCATAAGACGAATATCTAATATCAAAAATGTTACAATTCTGCTAAAATGTCTAAAAATAAAGATAATAATAAATAAATAAAAATATTGTGCAACACACAGAAACAAACCAAAAAAAAAAA

PERL-P6-H9 (SEQ ID NO:52)

CAAGATGAAAATCTTTCTGTGCCTAATTGCTGTGGTTTCCCTTCAGGGAGTTTTAGCTTATGATATTGAGAGGGAATACGCGTGGAAAAACATCAGTTTTGAAGGAATAGACCCAGCATCCTACAGCGTTAAAAATAGTATCGTAACTGGTTTCGCTCACGATGCAGATAGTAAGAAGATTTTCATTACTATTCCAAGGCTAAACCCAGTTCCGATTACTCTAACTGAACTGGATACCACTAAGCATCCGGAAGGATCTCCTCCACTAAGCAAATTTCCTGGTAGTGATAAATTAATCTCTGTTTATCAACCGGTCATTGACGAATGTCGCCGACTTTGGATTGTGGACGCTGGACAGGTTGAGTACAAAGGAGATGAGCAGAAGATTCCCAAGAAAAATGCTGCTATTATAGCTTATGATCTGACGAAGGACAATTATCCAGAAATTGATCGATACGAGATACCGAATAATGTTGCTGGTAATCCACTTGGATTTGGAGGATTTGCCGTTGATGTTACAAATCCGAAAGAGGGATGTGGTAAAACCTTTGTCTACATCACGAACTTCGAAGACAACACTCTAATAGTGTATGATCAGGAGAAGAAAGATTCCTGGAAGATCAGTCATGATTCATTCAAACCTGAGCATGAATCGATCCTGACCCACAACGGTGCTCAACACATTTTAAAGTTGGGTATATTCGGAATCACCTTAGGAGATCTGGATGAGGAGGGAAATCGTCAGGCTTACTACTTGGGAGGTAGTAGTACGAAGCTCTTTAGAGTGAACACCAAGGATCTCAAGAAGAAAGCCGGTCAAATTGAATTCACTCCTCTGGGAGATCGTGGATCTCACTCTGAAGCCCTTGCTCTGGCTTATGATCCCAAGAC

TAAAGTTATCTTTTTCATTGAATATAATTCTAAGCGAATCTCCTGCTGGA
ACACTCAGAAATCACTAAATCCTGACAACATTGATGTGATTTATCACAGT
CCTGATTTTATCTTCGGCACTGATATTTCAATGGATAGTGAATCCAAAT
TGTGGTTCTTTTCCAACGGTCATCCACCAATTGAGAATGTTCAACTAACT
TTTGATAAGCCACATTTTCGTCTTATAAGCATGGATACGAAAAAATCAAT
TCATGGTACTAAATGCGAAGTAAAACCTTAAGTCAAACTTGGAAAATAA
AACACTTCTTAAAGAAATTGTAATTTTTATGATGGTAATAAATTTTTGTG
TGCCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERL-P7-C2 (SEQ ID NO:54)

CACTTTAGTCTCAAATCTTGGATCATGTTTAAGAAATTTATCTTGGTGGC
CCTTGTCGTTGTCGTGGCACAATGTGCTCTTCCCGCAATCCCAATTGCAA
GACAGGGAAAAGATTTCCCCGTCCCGTTTGTAAGTGAAGATAATAATCC
GGATGATTATTTTGACGATCAGTACTATCCGGACATAAACGATGCGGGT
GTAGGTTCAAAGGCTCCGCAGGGAAGCAGAAAGCCACCCAATAGAGGC
ACCATCCCTCCTCCTCGTGGTGACCAAGTGTCATCTGGTGGACGAACTCC
ACCCGGAAGGGTTGGACAGGGTACAAGCCCTACAAAGGATAAAAGAGC
TCGTCCTCAGATTAACAGAAACCCAACCGGAACGGTTGGACAGGGTGGA
AGCCCTGGTACAAAGGATAAAAGAGCTCGTCCTCAGATTAACAGAAACC
CAACCGGAAGTGGTACAAAACCCAGAGATAGGGAGCTTGTGATTAGGG
ATAAGCCCCCATCCGGAAGTCAAGGTGGTAAACCTGGAAGACAGGTCAG
AGGCCCAAAGGAAGATTTGTCGCGTTATCAAAACGCTCCGGCAAAGTTG
ATTTTCAAATCGAGTAATATCAATACTGCTGGTAAAACCCCGAAGCGCT
GTGAAGTTGTTTAAGACGAAGAAGGACAAAACAGTTGTTGCTAAGGGAG
GTCCCAACGATGTTTATGAGGTGGAGCTTCTCGATGGAAATTTCAATAAT
ATGAGCTTGAGGATCCAGATAATGGACAGGAAGAGCAGCACAGCGATC
CTCAGCAATCCAGATCGCAACTTAATTGTTGGCCGTGTCAAGACGTACCG
CGGATTAAGATGAGGTGCTGAATTTTTAAATTTTATTTTATTTTTTTGCTC
CTAAATCCAAAATCCCCCCAAATAAATCAGTTTGAACGCAAAAAAAAAA
AAAAAAAAAAAAAAAAAAA

PERL-P6-H1 (SEQ ID NO:56)

GGCCATTACGGCCAGGGGGAATAAGTTAGTGTCTTCACGTTTATTGAAG
CTTTCACTTCAATATGACTTACTTCAAGATCAGTACTTGTTGTTTAGTTTT
AATAAGCCTCATTCTACCTATAATTTGTATTAAAGTTATTCGTTTTGATGA
TAGAGATGAATATCTTCTTGGTAAACCTGATAATACTGATGAAGAACTCC
TCTATTCAACCTTTGACTTCATTAAGAATACCTGCGCTAATCCTAAAATG
AAATGCACCAATAACGCCACTCATTTCGTTCTGGATTTCTCTGATCCGAA
GAAGAGATGTATCTCCTCCATCCATGTATTTTCCACTCCCGATGGACCTG
TTAATCTTGAGGAGGAGAATAAGCCTCGATCAAAGAGTTCAATTTACTG
CCAAGTGGGCGGCATTGGACAGAGTTACTGTTTGCTGGTGTTTAAAAAG
AAGGAACGTCGTGAGGATGCTCTGGTTGATATCCGGGGACTCAAAACAT
GCTCCCTCAAGGAGCGCTACACATCTGGAGATCCCAAGAAAACCGATGC
TTACGGAATGGCATACAAATTCGACAAGAATGATAATTGGAGCATCAAG
AGAGAAGGTGTTAAGCAATGGAAAAGATCAGGAAATGAGATCTTCTACC
GCAAGAATGGTTTGATGAACCATCAAATAAGATACTTGAGCAAGTTTGA
TAAGTACACGGTTACCAGAGAAATGGTCGTGAAGCACCGCGCTAAGAAA
TTCACCATGGACTTCTCCAACTATGGCCAGTACAGAATCAGTTTCTTGGA
CGTCTACTGGTTCCAGGAGTCCGTGAAGCACAAGCCGAAGTTACCCTAC
ATCTACTACAATGGCGAATGCTTGCCTAGCAATAAGACGTGTCAGTTGGT
TTTCGACGCTGATGAGCCTATTACTTATGCTTTTGTGAAAGTGTTCAGTA
ATCCGGACCACAACGAACCACGATTGAGGCATGCAGATCTGGGACGAGG
ATAGGAGTGGATTAGTCCGTTGTTGAAATTTGAATAAAATGCTATGAAG
ATGTTAAATTTGCCTCAAAAAAAAAAAAAAAAAAAAAAAAA

PERL-P3-E11 (SEQ ID NO:58)

AAAACATCTTCGCGTTTTCGTGCTATTTGAAACGGAGAACATCGAGTAA
AGAATATGAAGTTACTAATTACTATCGGTGCGGTTTGTGTGTTACAAGTC
GTTACAGTATCATCCATCTTCTTTCCCATTCCAATCAACATCCAAACAGG
GACGACATCATCATCATCAGGACAACCAGGACAGCAAGTTACAACGAGT
ATAAGTTTCAGTAATGTATCAAACATCACGGATATGGTGATTTATCTCAC
GCAGAATATCAGTAGAGCTCTCCTTACGCGTGTACCAAACCCTGATGAT
ATCAAATCAGCAGCGGATATCTTGGAAAGTTTTACAGGAAGCCTCAAGT

ATTTCCAAACACCTCCGGATGATGTGGATCAAGAGGAATCAGAGACAAA
GTCACGATCTAAGAGATCATTTACTGATATATTCAAACAATCTTCGCCTT
TAAAAGAAATCGGAGAAAGGATCGAAGAAATAAAAAAGAAACTAAAAG
GAATGCTCAAACCAAAACCGCAAACACCTTCTGGAAATCAAACTGATAG
CTCGAACACAACTTCGGAGACTCAATCGAGAAAGAAACGGGCTTTAACT
GACTTTATACCAATGGATTCTCTGAAAGATGCGATTTCAAAAACAGGGG
AAGTGTTGATACCTTCAAGTGCAAGTGCAAACTCTAGTCCTCTAGATTTT
ATGTCAAAACTATCCGATATCGCAAATGATCTTATTCAAAACTCAATGAA
GGAAATCTCCGAAAATTTAGCCTCAAGCGTTGCTATGTACCAAGTCAACT
CACAGTTAGATGCCATTAAACAATCCATGGATATTATAAAACAAGAAAT
TGATAAGACCCAAAAGATCCAGAAATACGTAAAGGAAGCTCTTAATCAA
GCCAAAAATGCTACTAAATCTTTAGGAGAAAAGCTTAAGTCCAGTAACT
GTTTCGCTCAATTTATAAATCCCTTTAAACTTTTTGAAAAAGGAATTACT
TGTGTGAAAAATAAAATCGATAATGGATTAAAAATCGCAAAAGACACCT
TTAAGAATTTACAACAGGCAATGAGTGTGCCCTCAGATATTCAAAGTGA
AGTGTCCAAATGCTCCCAAAATCAGCAATTGAATCCCATTGCCAAACTCC
TGTGCTACTTGAGGACACCACTGCAATTGGACGACGAGAAGTTGCTGCT
TCCCTTTGAATTTACGAGGAGAATTAGAGAAATAACTAACTATTTTGCCA
CCATGAGAATGGACCTCATTCGTTGTGGCATAGAAACTATTCAGTCGATC
GGAGACAAGGTTGAGGATTGTGCAAGAGAAGCAATATTGGCTGTAAAG
GACACTCTGAAGGGATAAAGTCCGCATTTTCTGGCTGTCCAATTGGGACT
AACCCAATCATTGATGATGCCGAGCTATTGTATGTTGGAGAAAATGAAT
AAAAGGCTTCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERL-P7-G12 (SEQ ID NO:60)

ATTAGAAAACCAATCATGAAGCAGCTTGTTGTATTTTTGGCGTTGATAGT
TCTGATAGTGATTTGTCACGCAGAACCACCTTCGAAGAAGTGTAGGAGT
GGACTGGTGAAAGATGAGGAGTGTATACTCCATTGTGAATACAAATACT
ATGGCTTTACTGATGATAATTTCGAACTTGATTCAGATCTAAGAGGACAC
TTTAGAACTGCTATGAGGAAGCACGGCGCAATTAGGATCGATCAGGAAA
GACAACTTGATAAGCATTTGAAAAAATGTGCTCAGGAAGCTAAAAAGTC
GGAAAAGTGTAGGAAAATCATTCAGTACTATCGCTGTGCTGTGAATAAT

AAACTTTTCCAATATAATGCTTATGCTAAAGCAATTATTGCGCTTGATAA
GACAATAAATGTTTAAAAAAGAAAGTGAAATGTATCTATCGCTCAAATA
AAGAAGGAAGCTAAGATCGTTGAAAGAAAAAAAAAAAAAAAAAAAAA
AAAAAAAA

PERL-P3-C9 (SEQ ID NO:62)

GCTTTAGAAGTTATTTTACATCTGTGCAATGATTAACTCAACAGTGATTC
AATTTATTTTTCTTTTTGTGATTTTTCTTCCTGGAAAATCTAAAAGTGCCC
CAAAGACTTGCGAAATTAATCTTCCCACCAGTATTCCGACAAAAGGTGA
ATCAATTTATCTTCTCAATGGAAATGGATCGGTCTTCCGACCGGATGGAA
AATTGACTCAACTCAATATTGGGGATTCCCTGTCCATCTACTGTCCTGGA
CAGAAGGAGCTCAAGAGAGTCCCTTGCAGTCCCAAATTTTCCCTTGAGA
ACATCACTTGCAACAGCAATGTTCACAGTGAATTGGTTGACACGGAGGA
AAAGTGCGGAAAAGATGGAAAATGTTACAATATTAGCTTTCCATTGCCA
ACAAATACCTTCCATACAATCTACAGAACTTGCTTCAACAAGCAGAAAC
TAACACCAATCTATTCTTATCACGTCATCAATGGAAAGGCAGTTGGATAT
CATGTGAAACAGCCACGAGGAAACTTTCGACCGGGAAAAGGTGTCTACA
GGAAGATCAACATCAATGAGCTCTACAAGACCCACATTTCGCGCTTCAA
GAGAATCATCGGATCCACCCAGACATTCTTCCGGAAGCCCCTGCACTATC
TGGCTCGTGGACATCTCTCACCTGAAGTGGACTTTGTCTTTGGCAACGAA
CAACACGCCACTGAGTTCTACATCAACACCGCCCCCAATATCAATCCAT
CAACCAGGGAAATTGGCTTCGAGTGGAGAAACACGTGCGCAAACTGGCC
AAGGCCCTCCAGGATGATCTCCACGTTGTCACTGGAATTTTGGGCATCCT
CAAGTTCTCAAACAAACGAGCCGAAAGAGAAATCTATCTGGGCGAAGG
AGTTATTCCTGTACCGCAAATATTTGGAAGGCTGTCTTCCACCCTAAAA
CCTCTTCCGCCATTGTCTTCGTGTCCTCTAACAACCCTCATGAGAAGACC
TTCAATCCAATGTGCAAGGATGTTTGTGAAACAGCAAGATTCGGAGGCA
AACAACATGAAAATCAAAATTTTTCCAATCACACAGTGGGATTCACCAT
CTGTTGTGAATTACCAGACTTTCTTGGAAACTCAAAAGTTATTCTTCCTA
AGGAGTTTCAAGGCAAAAACTACCGCAAGTTGCTTAAAATGCCAGGAAA
GCCATAAAAACTTTCATCTTATGGTGTTGTCACACGGCAATAGTTTTGAC
AACAGATCCTAGCTCAAACGGAATTCAATAGCATTTTCCTTTAGAAAACT

ATCATATTTTCATCGAAAAACAGTCTCTTACAATTCTGAGGATTTTTAAA
AAAGAATTTCAATTGAATCAGAATCTCTTTTAAGCACTGAAGAGAATCTC
CTGTCATTTTCTGATCTTCTATGGGTCTTTTCCAGAAAATTCTTGATTATT
CCTAAGAAGAAATTGATATTTAGTGAAGACTGTAATTGTTTAGCATTCAA
CAGTAAAAATTTGTTGACAGAGCTATAATTCCGTGTGACAACACCATTA
GTGGAAGACTCAACAAATCGATAAAAAAAATGATTTCAAAATGGTATAA
TAGTAAAAATAAAAACCTTTCCGGCAATAAATTATTCCTTTGAGGATCAC
AATGTCCTGAATATTCACACAGTGACTGAGTTTTAAGATTATTTTACTCT
CAAATCGTATAATAAAGGACAAAAAACATGCGTAAATAAAGAAATTTGC
AGTACGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERM-P2-A10 (SEQ ID NO:64)

CCATTACGGCCGAGGAGTCTCTTTCAACGCTTAATATCAGCAATGAATAA
CTTGTTAACATTCTTTGGAGTACTTTGCTTCTTGGGCTTTGCTAACTCTCT
GCGATTCCCTCGTGACCCAGACCAAACCAGATGGGCGGAAAAGACTTGT
CTGAGAGAATTTTCTCGTGCTCCACCTAGTCTTTTAAAGAAATGGCAACA
ACTGGACTTTCCCAATACCAATCTCACCCACTGCTTCATCAAGTGCTTCA
CTTCGTATCTTGGAGTCTACAACGACACGACTAAGAAATTTAACGTGGA
CGGAATTAAAACCCAATTTAAAAGTCAGGAAATTCCTGCACCTCAAGGT
CTTGAGACACTTCGTAAAACATCTAAAGGAACCTGCAAGGATATTTATCT
AATGACTGTGGACCTTGTCAAGAAAAACAAGCTACAATTCGCAAAAGCT
TTCCATGGAATTTCTGCAGAAGCTGCAAAATGGTATACCCAACATAAAG
GAAATGTTAAGGGAAAGTACCAGAAAGCATCGGAATTCTGCAAATCTAA
AGATGATGAGTGTAGGCTCCATTGCCGATTCTACTACTACCGCTTAGTTG
ACGAGGACTACCAGATATTCAACAGAAATTTAAAAATCAACGGTATTTC
CAACGCTCAACTTCAGCAATGCAGGAACAAAGCCAGTCAAGCTAAGGGT
TGCCAGGTGGCAAAGGTCCTAAGGCAATGTCTCAAAGACATTAATCCTG
AAAATGTAAAAGCGACTTTGAAGGAGTTGGATGAGATATCGGCGAAATA
ATATACTTAAATTAACCCCATCAGCCCAATTTAGCGTAATTTCTCGACCG
TAGAAAAAGGTGTTTAACTTACGGGTGATTGAGTGTAAGTAATTTAGCG
GCTGTGGGAGATGAAATGACTATTAAAAGGTTTATATCCCCAAAAAAAA
AAAAAAAAAAAAA

PERL-P6-H11 (SEQ ID NO:66)

AGTAAGTTTATCTGCGCGAGCGGAAATGGGTGCCATTTAGGCCGGAGTC
CAGTTAATATTCCGACATGTTGCAAATTAAACATTTCTTGTTCTTTGTGGT
GTTACTCGTGATCGTTCACGCTAACGACTATTGCCAGCCGAAATTGTGCA
CAAATGGCAAAACAGTGAAGCCTCACATTGGATGCAGGAATAATGGAG
ATTTCGATAGAAGTGCCTGTCCAAATGATGCTCAGATGGTTGAAATGACT
CAACAGAGGAAGGAGCTCTTTCTTAAGATTCACAATCGCCTTCGCGATA
GGTTCGCTCGTGGCTCAGTGCCCAATTTCAAGTCAGCCGCCAAGATGCCA
ATGCTGAAATGGGACAATGAATTGGCCAAGTTGGCAGAATACAATGTGA
GAACGTGCAAATTTGCTCACGATCAGTGTCGCGCAACCACAGCTTGTCCT
TATGCTGGTCAGAACTTGGGGCAAATGTTGTCATCTCCAGATTATTTGGA
CCCCGGCTATGCCATCAAGAATATCACCAGGGAGTGGTTCTTGGAGTAT
AAGTGGGCAGATCAACAACGTACCAACACCTTTACGGGAGGACCTGGTA
AAGATGGCAAACAAATTGGTCACTTTACTGCCTTCGTCCATGAGAAGAG
CGACAAGGTTGGATGTGCTGTTGCTAAATTAACGAACCGACAATTCAAC
ATGAAGCAGTACCTCATCGCTTGCAACTACTGCTACACGAATATGATGA
ACGAGAAGATCACAGCACAGGTGCCCCCGTTCTAAGTGCCAGAGTAAAA
AATGCGATTCCAAATACAAGAATTTGTGCGATGCCAGTGAGAAAGTCGA
AGCCATCCCAGACATCTTCCTCAAGAAGCGCAGGACATAATTCTCTGCTT
TCCCATTTGAAAATTGTAAAATAAATATTGTTTTCCCTTCTATCAGGTGA
ATTGGTGAAGATGAGAAGAAAGAATGTATAAGAAAATAAGAAATAAAC
AGAAACTGAGATATCGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERS-P1-H11 (SEQ ID NO:68)

ATCAGTTTCACTTTGACCATCGATGGTGAAATACTTCAATTCATTTTACG
AAATCACTCTGATTGAGAAACGATGATCGTGAAGGGTCTCCTTGGGGTG
TTTCTTGTGATCTTGCTCGTGTGCGTGACAGAACAGGGAGTGGACGGATA
CCACAGGGCTAATGGGGACTATGGTTACAGCTACGAAAACCGGCATCAC
GTAGTCAACGGAGATGAGGAGGAACATGAAATAAAACATACTAACTCTC
GTAAATTTGATGATGACGACTATCTCTTTAGTCACGGCTACGCCGCCTAC
GACGACGAAGACGATGAAGATGAACGACAGGGCTATTCAAGGGGGCGGT

GGGGGAGCCGGAGACAGTAGCAGAGATCCCGGATTTTATCGTCGTGGAA
GTCAGGAACAATCTTACGATCCCCACAGCGGTCAGACAGCTCCTGGCTA
CTCAGAATCCAGTGAATACGAACATAGCGGAGACTACGATAACTCCCAG
AACCAGCAATATTCCTCAACTCCCTCTAACGCTAACGTTAACCTAATCGA
CCAGTATCTCCATCTAATCCAATTACATAGCATCCCATCCGATTTAGTCC
AATACGCCGAATCCTACTTAACACACGCCAAGAACTCCATCCGATACTA
CGCCGTGCACGCCAAGGACTTTGAGAGGATTCGACCCTGCCTTGAATCC
GTCACGAAGTACTTCAATATGCTCAATGACGATCTCGCCAGGGAGTACG
TCAGATGTCAACGACAATGTTACCTTGATCGTCTCAATAGCTACACAACG
GCTATCTCTCAGTATACTGTCACCACAAATGCCTGCATAAACAACCGTTT
GAACTGAAGATGAGGCTTTTTTGTGAAATATTTATTTGGGTCAGTGAAA
ATAAATTTTCATCAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERM-P2-G11 (SEQ ID NO:70)

AGTATTCAGTTGTTAGAGATCTTTCCAACATGATATTGAAATTGTGCGCC
ATTGCGGTTTTATTTTTCCTTATTGGAGACGGAGAAGCAGCTCCTAGACC
AACAAGATTCATCCCTTTCGCTATCATCTCAGATCTGCACAGGAAGGCCA
TGCACGACGAAAAGAACAGATTTACTAGTATAGTGAAATATGGTCAATT
GAAGTACAATGGAGAGAAATATACTCTGTCCATCAGAAGTGAGAATCTC
CATTATTTCACAAAGGACACCTACAAAGGAACCGGAGCCGATATGTCCG
AGTTGATCTACTTCAATGACAAGCTCTACACTCTTAACGACGAAACAGG
AACTATCTATGAGGTGAAACACGGCGGAGAGCTCATTCCATGGATAACT
CTCAAGAATGACGATGGAAATCAAAAGGACGGCTTCAAAGCTAAATGG
GCAACAGTTAAGGGTAACAAGTTGATTGTCGGATCAGCAGGAATGGCCT
TTCTGGACGCGAAAACCATGAATATTGACAGAGACGCCCTCTGGGTGAA
GGAAATCAGCGAATCTGGCCACGTCACTAATAAATATTGGGATAGTCAA
TACAAGAAAGTGAGGGACGCCATGGGACTCGTCTCCGGATTTGTCTGGC
ATGAGGCCGTAAATTGGTCACCAAGGAAGAATCTTTGGGTCTTCATGCC
CAGGAAATGCACAAATGAACCATATACCGTTCGCTTAGACAAGAAAACC
GGATGCAATCAGATTATCACGGCCAATGAAAACTTCAATGATGTTAGAG
CAATTCATATCAATCGAGCCGCTGCAGATCCAGCTTCTGGATTCTCCTCT
TTCAAGTTCATCCCAAACACCAGAAACAATGATATCTTCGCAATCAAGA

CAATCGAGAGGAACGGCCAAACAGCCACTTATGGCACAGTGATTGACATCAATGGGAAGACTTTGTTGCCCGATCAGCGAATTCTCGATGATAAATATGAAGGAATTGCATTTTTCAAGGATCCCAAAGGAATTAAGTAAAGATGGATTATAAAATGTTGAAATAAAATGTCATGAAGCTTATAAAATGAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERM-P5-E2 (SEQ ID NO:72)

AGTTCAGTTTTCTGTGGAAAATGAATACCTTATTGAAAGTCGCGGTTTTGCTAAGCTTGGGAGGAACTGGGTACTCTTGGCAATATCCCAGGAATGCCGATCAAACTCTCTGGGCTTGGAGATCGTGTCAAAAGGAGCACATCGGCGACGACCAAGCATTATTGAAGAAATGGTTGAAATTTGAAATTCCAGATGATAAAGTAACGCATTGTTTTATTAAATGTACTTGGATCCATTTAGGAATGTACGATGAAAAAACTAAAACCATTAGGGTTGATAAGGTCAAGCAACAATTCGAGGGACGCAAATTACCAGTTCCTGCTGAAATCAGCAAATTAGAGGGTCCTACAGATGGCGATTGTGAAAAAATTTACAGAAAAACTAAGGCTTTTCTTGACGCTCAAATGAAGAATTATCGCATTGCATTCTATGGCATTTATGATGGATCCGATGCATGGTTTGCAGAACATCCCGAAACTAAGCCCAAGAAAACGAAGATTTCTGAATTCTGCAAAGGTCGTGAAGGTGGAAAGGAAGGAACTTGCAAGCATGCTTGCAGCATGTACTACTACCGCTTAGTCGATGAGGATAATCTTGTGATTCCCTTCAGGAAGTTGCCAGGCATCTCAGAGTCTGATCTTAAACAATGCAGAGATGCCGCTAGCAAGAAAAGTGGATGCCAAGTTGCTGATGACAATCTACGATTGTCTTAACAAGATCAACCCGACAGGTCTTAAAACTGCTTTAAATACGCTCGATGAGCAATCATTAACAAATTATTAGAAAAGAAATAAAAATTGATTTCGAGCAATCGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERM-P5-C11 (SEQ ID NO:74)

ATCATTAGTGAAGTTGTTAACAACTAAGCATGAAGTACTTTTCTCTCAATTTTCTTCTAATTGTGATTCTATTGATTGTGGCTTGTTCACCTCAATTACCATGTTTACCCCAGGATTCCAAGAAAAAGCCGTCCAATCCTCGTCCTAAATTATCGGCCAGAAGTGGTTTGTCTTATTGAGTTATCACACTAGGAATTCGATGCAGTAATTTATTACGTGGGCATTGTGGCTTCATAGCTGGGGCCGTAAAA

ATTAAAAGACAAAAAGAAATTATTACATGACGGCCGCCATAAGTCGACGAAAATGGACATAACATCCTTGACTACCTATCGTAATGTGAATTTGAAAAATTATACAAAAAAATAATTATGAATTAGCAAAAATAAAAATTATCAGAGGAGCAGATCTGCTGTTATGATTTCTTTTTATGTCTCTTTTATGTAAGCAATCACTATTCTTGTACGAATATATAAATAAAAGTTCCAACTGTGTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERM-P5-H8 (also referred to as P2-G9) (SEQ ID NO:76)

AGTCAGTTATTGTTCGAAAAATGAAGAAAATTGTGCTATTCAGTTTTATATTCGTTGCTTTGGTGATCAGTGCTAAAGCCATTGAGACGGAATTGGATGATCCCGATGATGCCACTAAAGGTCGGGATGTTGCGAAGGCAGAACCTGGACAACTGGGACAAGTTCCAGTTGTACCTGATTTAAATCCTTCGAACACGAGGAAACGGAGGAATAGATCCAGAAAAAGGCGACGAAATCTAGGAAAGAGACTCAAAAAAGTTTTTGCATAGAAATTAATACTAAAAAGATTAAAACTATGTCAATTTGATGCCTTTTGAGCATTCAATTAAAAAGTATGACAAATTATTAAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERL-P3-B3 (SEQ ID NO:78)

ACTTAATATTGGACTGTATTTTGAGATAGACACCCCAGAGTACGATGGTGCAATGTGAATTCGGTGGAACACCTTGTACGACTTTGAATATTTCATATCCAACGATCAAGCCACTGGTGAATGCCTGAGTGTTGTGTTGAGCTCAGTCGCGGTGGAGCAGCGAGCCGAGAAAGAATGGCAAAGGTGCAATAGAGATACTAAACTAGAGGAAAGACTTGAACGGTGACAGAGGAATAGGAGCAAGAAAGAAGTGTTGAGAATTTGCGGGAATTTCTATGGCCAATATTAAGTGTTGATTCAAAGAGTTTTCTACACAGAGAAATTGCGAGGTCACTTATTGGAAATCAATGAGAAAGTTTTTAATGTTTTTCGTGAAAGGAGTGAATAAAAATTGAGTGCTTTATACATGTGAGACTCCCCCTTTTCTGTGGAGAGACGATAAAAGGAAATTCGATATTTATGGGAAAAGTGATGAATTAGTGATACTGGTGGCTCTCGAAACACAAGTCACGAATTAGAAAACGTCCAAAGAGTGATTTTTGTGCTCTCCGGTGGCTGATATAAGAGAATGTGAAGAGTGAGGATGATGTCTCGCTGGAGCAAAAGTGTGAAATTTGTGTGCCTCCTCCTGTGTGGCGGG

TTCACGTTTCTCACAACATCAGCACGTGCCAAACCCACGCTGACCTTTCA
GCTTCCGCCCGCCCTCACGAACCTACCCCCCTTCATAGGCATCTCGCGAT
TTGTCGAACGCAAAATGCAGAATGACCAGATGAAGACCTACACTGGCGT
TCGGCAGACGAACGACTCTCTCGTGATGATCTACCACCATGATCTGACG
ATCGCCATCGTGGAATTGGGACCAGAGAAGACTCTCTTGGGTTGTGAAT
TGATAGAAATTAACAACGATGATGAAGGCGCCAAAGTGCTCACAGAACT
GGCCACCGTGAATATACCACTGCAGATCGACTTCCGGGAGATGGTGAAG
CTCATGAAGCAGTGCGAGAAGATCGATTACATGCGGAAAGTGAAACGCC
AGGGAGCATCAGAGAGTGACCAGACAACAAATCGTCAACATCAGACGG
GCTACTTTGGACTCGGAGGCGCCACCGCCGGTCTAAGCATCCTCAGTGG
CATCCTTCCCGGCACCAAGTGGTGTGGCACAGGAGACATCGCCAAAACA
TACCACGATCTCGGCACCGAGGCCACTATGGACATGTGCTGTCGCACTC
ATGATCTCTGTCCAGTGAAAGTGCGCTCATATCAGCAGCGCTACAATCTC
AGCAATAACTCTATCTACACAAAATCTCCCTGCAAATGTGATGACATGCT
GTTCAATTGCCTCAAGAGGACCAACACGTCAGCCTCGCAATTCATGGGG
ACCATCTACTTCAACGTGGTCCAAGTGCCATGTGTTCTGGACACAGAGA
GAGGCTACAGATTCAGAAAAGCGAGAACCTTCTCCTGAGTATTGCAAAA
CAACGAAATCTGCGGATTTTTTTTATTTTTGGGACTTTTCGTGTGTAAAG
ACCATTTCTTGTGATTTTCAGCTGCGGTGCTCTTTCAAATGAATTATTTAT
GTTGCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERM-P2-D11 (SEQ ID NO:80)

GTATTAGAAAACCAATCATGAAGCAGCTTGTTGTATTTTTGGCGTTGATA
GTTCTAATAGTGATTTGTCACGCAAAACGACCTTCGAGGAAGTGTAGGA
GTGGAATGGTGAAAGAGGAAGAGTGTATACTCCATTGTGAGTACAAATA
TTATGGCTTTACCGATGATAAGTTCCAACTTGATGCAGATCAGAGAGGA
AACTTTAGATTTGCCATGATGGACTATGGAGCAATTAGGATGGATCAGG
AGGGTCAAATGGATGAGCATTTGAAAAAATGTGCCAATGAAGCTGAAAA
GGCTCCAGTGTGCTCCAAGGTGGATAAGTGTAGGAAAATCATTCAGTAC
TATCGCTGTGCAGTGAATAATAAACTTTTCCAATATAATGCTTATGCCAA
AGCAATTATTGCGCTTGATAAGACAATTAATGTTTAAAAAGTGGAATGA

ATCCCTAAAATAAAGAAGGAAAGATAAGAACTTTCAAGAAAACTTGAA
AAAAAAAAAAAAAAAAAAAAAAAAAA

PERM-P5-E3 (SEQ ID NO:82)

ACACACATACGATTCATTACCAGAAATGAAGCAGTTACCAGTGATCCTT
CTGGCCTTAGTCTTTCTGATCGCAAAATGTCGATCAGAAAAACCGGAAT
ATAAGTGCCGCAGAGACTTCAAGACCGAGGATAAAAATTGCTTCCTTTC
TTGTACATTTAAAAATTACCACTTCATTGATAACAAGTTCAGGATTGAAA
GGAAGAATATTGAAAACTACAAGAAGTTCATAACTGACTATAAGGCCCT
GAAACCCAATGTTAGCGATAATGATTTGGAAAAACACCTGTTGGATTGT
TGGGATAAATTCCAAAAATCACCTGAAGCATCAACAAGGCCCGAAAAAT
GTGAAAAAGTCAACAACTTTGAAAGATGTGTTATTGACAAGAATATCTT
TGATTATCCTATTTACTTCAATGCTTTGAAGAAAATAAATTACATTACAA
AGGTTTAATGAAAAATTGATGAAATAAACATAATGAATTATTGCATTGA
ATAACAAAAAAAAAAAAAAAAAAAAAAAAAAAA

PERM-P2-F11 (SEQ ID NO:84)

AGTTATTGTTGGGAAAATGAAGAAAATTGTGCTGTTCAGTGTTATATTCA
TTGCTTTGGTGATCAGTGCTAAAGCCATTG
AGGATGAGGATGATGATGATGACGATGATGAATCTGAAGATCGG
GATGTTGCGAGGGCAGAACGTGAACAACAGGAAGAAGAACCAGACGAA
CCTGAATATATTCCTTCTAGACCGAGGAATCGGTCGAAAATGAGAAAAT
GGAGGAATAGAAACTATAGAAAATATAGAGACGAAAGTAGGAAAAGAA
AGCGAGATATGGTTTTGGATGTTATCAGAAGATTTTTATAGAAATTAATA
CTAAAAGTATTAAGTGGATCAATTTGATGCCTTTTGAGTGATTCATTTTG
AACTTTGAAAAATAAAACAAAGAATGTAAAAAAAAAAAAAAAAAAAAA
AAAAAAAA

Also included are fragments of the above-described nucleic acid sequences that are at least 33 bases, at least 36 bases, at least 42 bases or at least 48 bases in length, which is sufficient to permit the fragment to selectively hybridize to a polynucleotide that encodes a disclosed *P. ariasi* polypeptide or that encodes a disclosed *P. perniciosus* polypeptide under physiological conditions. The term

"selectively hybridize" refers to hybridization under moderately or highly stringent conditions, which excludes non-related nucleotide sequences.

Also disclosed herein are open reading frames (ORFs) encoding a *P. ariasi* or a *P. perniciosus* polypeptide. These ORFs are delimited by a start codon and by a stop codon. This also includes the degenerate variants and nucleotide sequences encoding conservative variants and homologs. Specific, non-limiting examples of ORFs encoding a *P. ariasi* or a *P. perniciosus* polypeptide include a nucleic acid sequence from position 104 to position 1444 (104-1444) of SEQ ID NO:2, 4-759 of SEQ ID NO:4, 23-1189 of SEQ ID NO:6, 19-1200 of SEQ ID NO:8, 23-901 of SEQ ID NO:10, 25-963 of SEQ ID NO:12, 21-1148 of SEQ ID NO:14, 22-438 of SEQ ID NO:16, 24-1190 of SEQ ID NO:18, 29-787 of SEQ ID NO:20, 34-375 of SEQ ID NO:22, 19-852 of SEQ ID NO:24, 73-822 of SEQ ID NO:26, 40-1041 of SEQ ID NO:28, 29-442 of SEQ ID NO:30, 53-565 of SEQ ID NO:32, 29-781 of SEQ ID NO:34, 45-182 of SEQ ID NO:36, 85-783 of SEQ ID NO:38, 26-223 of SEQ ID NO:40, 69-413 of SEQ ID NO:42, 70-945 of SEQ ID NO:44, 19-438 of SEQ ID NO:46, 15-428 of SEQ ID NO:48, 46-1227 of SEQ ID NO:49, 5-1171 of SEQ ID NO:51, 25-651 of SEQ ID NO:53, 63-995 of SEQ ID NO:55, 55-1350 of SEQ ID NO:57, 16-411 of SEQ ID NO:59, 29-1195 of SEQ ID NO:61, 43-792 of SEQ ID NO:63, 66-776 of SEQ ID NO:65, 73-846 of SEQ ID NO:67, 30-1025 of SEQ ID NO:69, 21-713 of SEQ ID NO:71, 30-179 of SEQ ID NO:73, 21-269 of SEQ ID NO:75, 584-1465 of SEQ ID NO:77, 18-431 of SEQ ID NO:79, 26-451 of SEQ ID NO:81, 17-310 of SEQ ID NO:83.

In several embodiments:

The PRL-P4-A10 mature protein is 426 amino acids long (21-446 of SEQ ID NO:1) and is encoded by the nucleic acid sequence 164-1441 of SEQ ID NO:2.

The PRL-P4-A9 mature protein is 232 amino acids long (20-251 of SEQ ID NO:3) and is encoded by the nucleic acid sequence 61-756 of SEQ ID NO:4.

The PRL-P4-C10 mature protein is 370 amino acids long (19-388 of SEQ ID NO:5) and is encoded by the nucleic acid sequence 77-1186 of SEQ ID NO:6.

The PRL-P4-D6 mature protein is 375 amino acids long (19-393 of SEQ ID NO:7) and is encoded by the nucleic acid sequence 73-1197 of SEQ ID NO:8.

The PRL-P4-D7 mature protein is 275 amino acids long (18-292 of SEQ ID NO:9) and is encoded by the nucleic acid sequence 74-898 of SEQ ID NO:10.

The PRL-P4-E5 mature protein is 290 amino acids long (23-312 of SEQ ID NO:11) and is encoded by the nucleic acid sequence 91-960 of SEQ ID NO:12.

The PRL-P4-F3 mature protein is 350 amino acids long (26-375 of SEQ ID NO:13) and is encoded by the nucleic acid sequence 96-1145 of SEQ ID NO:14.

The PRL-P4-G12 mature protein is 118 amino acids long (21-138 of SEQ ID NO:15) and is encoded by the nucleic acid sequence 82-435 of SEQ ID NO:16.

The PRL-P4-G7 mature protein is 365 amino acids long (24-388 of SEQ ID NO:17) and is encoded by the nucleic acid sequence 93-1187 of SEQ ID NO:18.

The PRL-P6-E11 mature protein is 234 amino acids long (19-252 of SEQ ID NO:19) and is encoded by the nucleic acid sequence 83-784 of SEQ ID NO:20.

The PRM-P3-A6 mature protein is 93 amino acids long (21-113 of SEQ ID NO:21) and is encoded by the nucleic acid sequence 94-372 of SEQ ID NO:22.

The PRM-P3-F11 mature protein is 258 amino acids long (20-277 of SEQ ID NO:23) and is encoded by the nucleic acid sequence 76-849 of SEQ ID NO:24.

The PRM-P5-D6 mature protein is 228 amino acids long (22-249 of SEQ ID NO:25) and is encoded by the nucleic acid sequence 136-819 of SEQ ID NO:26.

The PRM-P5-E9 mature protein is 313 amino acids long (21-333 of SEQ ID NO:27) and is encoded by the nucleic acid sequence 100-1038 of SEQ ID NO:28.

The PRM-P5-F12 mature protein is 116 amino acids long (22-137 of SEQ ID NO:29) and is encoded by the nucleic acid sequence 92-439 of SEQ ID NO:30.

The PRM-P5-F2 mature protein is 150 amino acids long (21-170 of SEQ ID NO:31) and is encoded by the nucleic acid sequence 113-562 of SEQ ID NO:32.

The PRM-P5-G11 mature protein is 231 amino acids long (20-250 of SEQ ID NO:33) and is encoded by the nucleic acid sequence 86-778 of SEQ ID NO:34.

The PRM-P5-H4 mature protein is 21 amino acids long (25-45 of SEQ ID NO:35) and is encoded by the nucleic acid sequence 117-179 of SEQ ID NO:36.

The PRS-P1-B11 mature protein is 215 amino acids long (18-232 of SEQ ID NO:37) and is encoded by the nucleic acid sequence 136-780 of SEQ ID NO:38.

The PRS-P1-B4 mature protein is 45 amino acids long (21-65 of SEQ ID NO:39) and is encoded by the nucleic acid sequence 86-220 of SEQ ID NO:40.

The PRS-P1-E7 mature protein is 93 amino acids long (22-114 of SEQ ID NO:41) and is encoded by the nucleic acid sequence 132-410 of SEQ ID NO:42.

The PRS-P1-G9 mature protein is 262 amino acids long (30-291 of SEQ ID NO:43) and is encoded by the nucleic acid sequence 157-942 of SEQ ID NO:44.

The PRS-P2-C8 mature protein is 119 amino acids long (21-139 of SEQ ID NO:45) and is encoded by the nucleic acid sequence 79-435 of SEQ ID NO:46.

The PRS-P2-G8 mature protein is 118 amino acids long (20-137 of SEQ ID NO:47) and is encoded by the nucleic acid sequence 72-425 of SEQ ID NO:48.

The PERL-P7-G8 mature protein is 375 amino acids long (19-393 of SEQ ID NO:49) and is encoded by the nucleic acid sequence 100-1224 of SEQ ID NO:50.

The PERL-P6-H9 mature protein is 370 amino acids long (19-388 of SEQ ID NO:51) and is encoded by the nucleic acid sequence 59-1168 of SEQ ID NO:52.

The PERL-P7-C2 mature protein is 191 amino acids long (18-208 of SEQ ID NO:53) and is encoded by the nucleic acid sequence 76-648 of SEQ ID NO:54.

The PERL-P6-H1 mature protein is 282 amino acids long (29-310 of SEQ ID NO:55) and is encoded by the nucleic acid sequence 147-992 of SEQ ID NO:56.

The PERL-P3-E11 mature protein is 411 amino acids long (21-431 of SEQ ID NO:57) and is encoded by the nucleic acid sequence 115-1347 of SEQ ID NO:58.

The PERL-P7-G12 mature protein is 112 amino acids long (20-131 of SEQ ID NO:59) and is encoded by the nucleic acid sequence 73-408 of SEQ ID NO:60.

The PERL-P3-C9 mature protein is 365 amino acids long (24-388 of SEQ ID NO:61) and is encoded by the nucleic acid sequence 98-1192 of SEQ ID NO:62.

The PERM-P2-A10 mature protein is 230 amino acids long (20-249 of SEQ ID NO:63) and is encoded by the nucleic acid sequence 100-789 of SEQ ID NO:64.

The PERL-P6-H11 mature protein is 217 amino acids long (20-236 of SEQ ID NO:65) and is encoded by the nucleic acid sequence 123-773 of SEQ ID NO:66.

The PERS-P1-H11 mature protein is 232 amino acids long (26-257 of SEQ ID NO:67) and is encoded by the nucleic acid sequence 148-843 of SEQ ID NO:68.

The PERM-P2-G11 mature protein is 311 amino acids long (21-331 of SEQ ID NO:69) and is encoded by the nucleic acid sequence 90-1022 of SEQ ID NO:70.

The PERM-P5-E2 mature protein is 211 amino acids long (20-230 of SEQ ID NO:71) and is encoded by the nucleic acid sequence 78-710 of SEQ ID NO:72.

The PERM-P5-C11 mature protein is 24 amino acids long (26-49 of SEQ ID NO:73) and is encoded by the nucleic acid sequence 105-176 of SEQ ID NO:74.

The PERM- P5-H8 (also referred to P2-G9) mature protein is 62 amino acids long (21-82 of SEQ ID NO:75) and is encoded by the nucleic acid sequence 81-266 of SEQ ID NO:76.

The PERL-P3-B3 mature protein is 264 amino acids long (30-293 of SEQ ID NO:77) and is encoded by the nucleic acid sequence 671-1462 of SEQ ID NO:78.

The PERM-P2-D11 mature protein is 118 amino acids long (20-137 of SEQ ID NO:79) and is encoded by the nucleic acid sequence 75-428 of SEQ ID NO:80.

The PERM-P5-E3 mature protein is 121 amino acids long (21-141 of SEQ ID NO:81) and is encoded by the nucleic acid sequence 86-448 of SEQ ID NO:82.

The PERM-P2-F11 mature protein is 77 amino acids long (21-97 of SEQ ID NO:83) and is encoded by the nucleic acid sequence 77-307 of SEQ ID NO:84.

Another specific, non-limiting example of a polynucleotide encoding a *P. ariasi* polypeptide is a polynucleotide having at least 75%, 85%, 90%, 95%, or 99% homologous to one of the sequences set forth above that encodes a polypeptide having an antigenic epitope or function of a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide. Yet another specific, non-limiting example of a polynucleotide encoding a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide is a polynucleotide that encodes a polypeptide that is specifically bound by an antibody that specifically binds the *P. ariasi* polypeptide or the *P. perniciosus* polypeptide.

The *P. ariasi* polynucleotides and *P. perniciosus* polynucleotides include a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of DNA.

Recombinant vectors are also disclosed herein that include a polynucleotide encoding a polypeptide or a fragment thereof according to the disclosure.

Recombinant vectors include plasmids, viral vectors, and bacterial vectors and may be used for *in vitro* or *in vivo* expression.

A plasmid may include a DNA transcription unit, a nucleic acid sequence that permit it to replicate in a host cell, such as an origin of replication (prokaryotic or eukaryotic). A plasmid may also include one or more selectable marker genes and other genetic elements known in the art. Circular and linear forms of plasmids are encompassed in the present disclosure.

For *in vivo* expression, the promoter is generally of viral or cellular origin. In one embodiment, the cytomegalovirus (CMV) early promoter (CMV-IE promoter), including the promoter and enhancer, is of use. The CMV-IE promoter can be of human or murine origin, or of other origin such as rat or guinea pig (see EP 0260148; EP 0323597; WO 89/01036; Pasleau *et al.*, *Gene* **38**:227-232, 1985; Boshart M. *et al.*, *Cell* 41:521-530, 1985). Functional fragments of the CMV-IE promoter may also be used (WO 98/00166). The SV40 virus early or late promoter and the Rous Sarcoma virus LTR promoter are also of use. Other promoters include but are not limited to, a promoter of the cytoskeleton gene, such as (but not limited to) the desmin promoter (Kwissa M. *et al.*, *Vaccine* **18(22)**:2337-2344, 2000), or the actin promoter (Miyazaki J. *et al.*, *Gene* **79(2)**:269-277, 1989). When several genes are present in the same plasmid, they may be provided in the same transcription unit or in different units.

The plasmids may also comprise other transcription regulating elements such as, for example, stabilizing sequences of the intron type. In several embodiments the plasmids include the first intron of CMV-IE (WO 89/01036), the intron II of the rabbit β-globin gene (van Ooyen *et al.*, *Science* **206**: 337-344, 1979), the signal sequence of the protein encoded by the tissue plasminogen activator (tPA; Montgomery *et al.*, *Cell. Mol. Biol.* **43**:285-292, 1997), and/or a polyadenylation signal (polyA), in particular the polyA of the bovine growth hormone (bGH) gene (US-A-5,122,458) or the polyA of the rabbit β-globin gene or of SV40 virus.

In a specific, non-limiting example, the pVR1020 plasmid (VICAL Inc.; Luke C. *et al.*, *Journal of Infectious Diseases* **175**:91-97, 1997; Hartikka J. *et al.*, *Human Gene Therapy* **7**:1205-1217, 1996)) can be utilized as a vector for the insertion of such a polynucleotide sequence, generating recombinant plasmids such

as, but not limited to, PJV001, PJV002, PJV003, PJV004, PJV005, PJV006, PJV007, PJV008, PJV009, PJV010, PJV011, PJV012, PJV013, PJV014, PJV015, PJV016, PJV017, PJV018, PJV019, PJV020, PJV021, PJV022, PJV023, PJV024, PJV025, PJV026, PJV027, PJV028, PJV029, PJV030, PJV031, PJV032, PJV033, PJV034, PJV035, PJV036, PJV037, PJV038, PJV039, PJV040, PJV041, or PJV042. The plasmids are evaluated in dogs in order to determine their efficacy against a Leishmania infection (Vidor E. et al., P3.14, XXIV World Veterinary Congress, Rio de Janeiro, Brazil, 18-23 August 1991).

Various viral vectors are also of use with a polynucleotide encoding a *P. ariasi* or a *P. perniciosus* polypeptide. A specific, non-limiting example includes recombinant poxvirus, including avipox viruses, such as the canarypox virus. Another specific, non-limiting example includes vaccinia viruses (U.S. Patent No. 4,603,112), such as attenuated vaccinia virus such as NYVAC (see U.S. Patent No. 5,494,807) or Modified Vaccinia virus Ankara (MVA, Stickl H. and Hochstein-Mintzel V., *Munch. Med. Wschr.* **113**:1149-1153, 1971; Sutter G. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**:10847-10851, 1992; Carroll M. W. *et al.*, *Vaccine* **15(4)**:387-394, 1997; Stittelaar K. J. *et al.*, *J. Virol.* **74(9)**:4236-4243, 2000; Sutter G. *et al.*, *Vaccine* **12(11)**:1032-1040, 1994). When avipox viruses are used, canarypox viruses (U.S. Patent No. 5,756,103) and fowlpox viruses (U.S. Patent No. 5,766,599) are of use, such as attenuated viruses. For recombinant canarypox virus vectors, the insertion sites may be in particular in the ORFs C3, C5 or C6. When the expression vector is a poxvirus, the heterologous polynucleotide can be inserted under the control of a poxvirus specific promoter, such as the vaccinia virus 7.5kDa promoter (Cochran *et al.*, *J. Virology* **54**:30-35, 1985), the vaccinia virus I3L promoter (Riviere *et al.*, *J. Virology* **66**:3424-3434, 1992), the vaccinia virus HA promoter (Shida, *Virology* **150**:451-457, 1986), the cowpox virus ATI promoter (Funahashi *et al.*, *J. Gen. Virol.* **69**:35-47, 1988), other vaccinia virus H6 promoter (Taylor *et al.*, *Vaccine* **6**:504-508, 1988; Guo *et al.*, *J. Virol.* **63**:4189-4198, 1989; Perkus *et al.*, *J. Virol.* **63**:3829-3836, 1989).

Other viral vectors of use are herpes virus or adenovirus vectors. Specific, non-limiting examples include a canine herpes virus (CHV) or canine adenovirus (CAV) vector (for example, see U.S. Patent No. 5,529,780; U.S. Patent No.

5,688,920; Published PCT Application No. WO 95/14102). For CHV, the insertion sites may be in particular in the thymidine kinase gene, in the ORF3, or in the UL43 ORF (see U.S. Patent No. 6,159,477). For CAV, the insertion sites may be in particular in the E3 region or in the region located between the E4 region and the right ITR region (see U.S. Patent No. 6,090,393; U.S. Patent No. 6,156,567). In one embodiment in CHV or CAV vectors the insert is in general under the control of a promoter (as described above for the plasmids), such as CMV-IE promoter.

Multiple insertions can be done in the same vector using different insertion sites or using the same insertion site. When the same insertion site is used, each polynucleotide insert is inserted under the control of different promoters. The insertion can be done tail-to-tail, head-to-head, tail-to-head, or head-to-tail. IRES elements (Internal Ribosome Entry Site, see European Patent EP 0803573) can also be used to separate and to express multiple inserts operably linked to the same promoter. Bacterial vectors may also be used for *in vivo* expression.

Any polynucleotide according to the disclosure can be expressed *in vitro* by DNA transfer or expression vectors into a suitable host cell. The host cell may be prokaryotic or eukaryotic. The term "host cell" also includes any progeny of the subject host cell. Methods of stable transfer, meaning that the foreign polynucleotide is continuously maintained in the host cell, are known in the art. Host cells can include bacteria (e.g. *Escherichia coli*), yeast, insect cells, and vertebrate cells. Methods of expressing DNA sequences in eukaryotic cells are well known in the art.

As a method for *in vitro* expression, recombinant Baculovirus vectors (*e.g.*, Autographa California Nuclear Polyhedrosis Virus (AcNPV)) can be used with the nucleic acids disclosed herein. For example, polyhedrin promoters can be utilized with insect cells (for example Spodoptera frugiperda cells, like Sf9 available at the ATCC under the accession number CRL 1711, Sf21) (see for example, Smith *et al.*, *Mol. Cell Biol.* 3:2156-2165, 1983; Pennock *et al.*, *Mol. Cell Biol.* 4: 399-406, 1994; Vialard *et al.*, *J. Virol.* 64:37-50, 1990; Verne A., *Virology* 167:56-71, 1988; O'Reilly *et al.*, "Baculovirus expression vectors, A laboratory manual," New York Oxfore, Oxfore University Press, 1994; Kidd I. M. & Emery V.C., "The use of baculoviruses as expression vectors," *Applied Biochemistry and Biotechnology*

42:37-159, 1993; European Patent No. EP 0370573; European Patent No. EP 0265785; U.S. Patent No. 4,745,051). For expression the BaculoGold ™ Starter Package (Cat # 21001K) from Pharmingen (Becton Dickinson) can be used.

As a method for *in vitro* expression, recombinant *E. coli* can be used with a vector. For example, when cloning in bacterial systems, inducible promoters such as arabinose promoter, pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter), and the like may be used.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method using procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

When the host is a eukaryote, methods of transduction of DNA such as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with *P. ariasi* polynucleotide sequences or *P. perniciosus* polynucleotide sequences, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector (see above), such as a herpes virus or adenovirus (for example, canine adenovirus 2), to transiently transduce eukaryotic cells and express the protein (see for example, Eukaryotic Viral Vectors, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of recombinantly expressed polypeptides may be carried out by conventional means including preparative chromatography (*e.g.*, size exclusion, ion exchange, affinity), selective precipitation and ultra-filtration. Such a recombinantly expressed polypeptide is part of the present disclosure. The methods for production of such a polypeptide are also encompassed, in particular the use of a host cell and a recombinant expression vector comprising a polynucleotide according to the disclosure.

Antibodies

A polypeptide of the disclosure or a fragment thereof according to the disclosure can be used to produce antibodies. Polyclonal antibodies, antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibodies are included. Without being bound by theory, antibodies are of use as markers for exposure, and as immunodiagnostic tools to follow the development of the immune response to Phlebotomus salivary proteins.

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green *et al.*, "Production of Polyclonal Antisera," *Immunochemical Protocols, pp.* 1-5, Manson, ed., *Humana Press,* 1992; Coligan *et al.*, "Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters," *Current Protocols in Immunology,* section 2.4.1, 1992.

The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, *Nature* **256**:495, 1975; Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al., Antibodies: A Laboratory Manual*, p. 726, Cold Spring Harbor Pub., 1988. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, *e.g.*, Coligan *et al.*, sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes *et al.*, "Purification of Immunoglobulin G (IgG)," *Methods in Molecular Biology*, Vol. 10, pages 79-104, Humana Press, 1992.

Methods of *in vitro* and *in vivo* multiplication of monoclonal antibodies are well known to those skilled in the art. Multiplication *in vitro* may be carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally supplemented by a mammalian serum such as fetal calf serum or

trace elements and growth-sustaining supplements such as normal mouse peritoneal exudate cells, spleen cells, thymocytes, or bone marrow macrophages. Production *in vitro* provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large-scale hybridoma cultivation can be carried out by homogenous suspension culture in an airlift reactor, in a continuous stirrer reactor, or in immobilized or entrapped cell culture. Multiplication *in vivo* may be carried out by injecting cell clones into mammals histocompatible with the parent cells, *e.g.*, syngeneic mice, to cause growth of antibody-producing tumors. In one embodiment, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

Antibodies can also be derived from subhuman primates. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in WO 91/11465, 1991, and Losman *et al.*, *Int. J. Cancer* **46**:310, 1990.

Alternatively, an antibody that specifically binds a polypeptide can be derived from a humanized monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l Acad. Sci. USA* **86**:3833, 1989. Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* **321**:522, 1986; Riechmann *et al.*, *Nature* **332**:323, 1988; Verhoeyen *et al.*, *Science* **239**:1534, 1988; Carter *et al.*, *Proc. Nat'l Acad. Sci. USA* **89**:4285, 1992; Sandhu, *Crit. Rev. Biotech.* **12**:437, 1992; and Singer *et al.*, *J. Immunol.* **150**:2844, 1993.

Antibodies can be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, p. 119, 1991; Winter *et al.*, *Ann. Rev. Immunol.* **12**:433, 1994. Cloning and expression vectors that are useful for

producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibodies can be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.*, *Nature Genet.* **7**:13, 1994; Lonberg *et al.*, *Nature* **368**:856, 1994; and Taylor *et al.*, *Int. Immunol.* **6**:579, 1994.

Antibodies include intact molecules as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv which are capable of binding the epitopic determinant. These antibody fragments retain some ability to selectively bind with their antigen or receptor and are defined as follows:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain (L) and a portion of one heavy chain(H);

(2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) $(Fab')_2$, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; $F(ab')_2$ is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain (V_L) and the variable region of the heavy chain (V_H) expressed as two chains; and

(5) Single chain antibody (SCA), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art. (See for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1988).

Antibody fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and in one embodiment, a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Patents No. 4,036,945 and No. 4,331,647, and references contained therein; Nisonhoff *et al.*, *Arch. Biochem. Biophys.* **89**:230, 1960; Porter, *Biochem. J.* **73**:119, 1959; Edelman *et al.*, *Methods in Enzymology*, Vol. 1, page 422, Academic Press, 1967; and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent (Inbar *et al.*, *Proc. Nat'l Acad. Sci. USA* 69:2659, 1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. *See, e.g.*, Sandhu, *supra*. In one embodiment, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences

encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are known in the art (see Whitlow *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 97, 1991; Bird *et al.*, *Science* **242**:423, 1988; U.S. Patent No. 4,946,778; Pack *et al.*, *Bio/Technology* **11**:1271, 1993; and Sandhu, *supra)*.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (Larrick *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 106, 1991).

Antibodies can be prepared using an intact polypeptide or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or a peptide used to immunize an animal can be derived from substantially purified polypeptide produced in host cells, *in vitro* translated cDNA, or chemical synthesis which can be conjugated to a carrier protein, if desired. Such commonly used carriers which are chemically coupled to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize an animal (*e.g.*, a mouse, a rat, or a rabbit).

Polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (See for example, Coligan *et al.*, Unit 9, *Current Protocols in Immunology*, Wiley Interscience, 1991).

It is also possible to use the anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the

hypervariable region that is the "image" of the epitope bound by the first monoclonal antibody.

In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, label (*e.g.*, enzymes or fluorescent molecules) drugs, toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide.

In one embodiment, an antibody that binds a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide can be used to assess whether a subject has been bitten by a sand fly. In one specific, non-limiting example, a sample is obtained from a subject of interest, such as a human or a dog. The sample can be a body fluid (*e.g.*, blood, serum, urine, saliva, etc.) or a tissue biopsy. The sample or a fraction thereof is contacted with the antibody, and the ability of the antibody to form an antigen-antibody complex is assessed. One of skill in the art can readily detect the formation of an antigen-antibody complex. For example, ELISA or radio-immune assays can be utilized.

Immunogenic Compositions, Vaccines and Methods of Use

Immunogenic compositions and vaccines are disclosed herein. In one embodiment the immunogenic compositions and vaccines include a polypeptide. In another embodiment, the immunogenic compositions and vaccines include a recombinant vector, such as a viral vector or a plasmid. When administered to a subject such an immunogenic composition or vaccine generates an immune response to the sand fly's salivary protein(s), and surprisingly a reduction of the leishmaniasis symptoms and a decrease of the leishmania parasite load. Thus, without being bound by theory, a cellular response, such as a Th1 response, produced to the salivary protein can indirectly kill a Leishmania parasite. For example, a Th1 type response can allow macrophages to take up Leishmania antigens and present them to T cells in a Th1 context. The induction of the Th1 response can produce an anti-Leishmania immune response, or can prime the immune system of the mammalian host for anti-Leishmania immunity in response to a later infection.

In one embodiment, the immunogenic composition or the vaccine includes an effective amount of at least one *P. ariasi* polypeptide disclosed herein. The immunogenic composition and the vaccine can include a pharmaceutically acceptable excipient and/or an adjuvant. In one embodiment, the immunogenic composition or vaccine includes a polypeptide having an amino acid sequence as set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, or SEQ ID NO:47, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In another embodiment, the composition includes a polypeptide having a sequence as set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:29, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:39, or SEQ ID NO:43, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In another embodiment, the immunogenic composition or vaccine includes a polypeptide having an amino acid sequence as set forth as SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, or SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In yet another embodiment, the immunogenic composition or vaccine includes a polypeptide having an amino acid sequence as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, or SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In a particular embodiment the immunogenic composition or vaccine comprises the five polypeptides as set forth as SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, and SEQ ID NO:39. In a particular embodiment the immunogenic composition or vaccine comprises the four polypeptides as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, and SEQ ID NO:39.

In another embodiment, the immunogenic composition or the vaccine includes an effective amount of at least one *P. perniciosus* polypeptide disclosed

herein. The immunogenic composition or the vaccine can include a pharmaceutically acceptable excipient and/or an adjuvant. In one embodiment, the immunogenic composition or vaccine includes a polypeptide having an amino acid sequence as set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In another embodiment, the immunogenic composition or vaccine includes a polypeptide having a sequence as set forth as SEQ ID NO:55, SEQ ID NO:63, SEQ ID NO:73, or SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In yet another embodiment, the immunogenic composition or vaccine includes a polypeptide having an amino acid sequence as set forth as SEQ ID NO:73, or SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In a particular embodiment the immunogenic composition or vaccine comprises the four polypeptides as set forth as SEQ ID NO:55, SEQ ID NO:63, SEQ ID NO:73, and SEQ ID NO:75. In another particular embodiment the immunogenic composition or vaccine comprises the two polypeptides as set forth as SEQ ID NO:73 and SEQ ID NO:75. In another particular embodiment the immunogenic composition or vaccine comprises the polypeptide as set forth as SEQ ID NO:75.

In a further embodiment, the immunogenic compositions and the vaccines may comprise a combination including at least one *P. ariasi* polypeptide disclosed herein and at least one *P. perniciosus* polypeptide disclosed herein. In one embodiment, the immunogenic composition or vaccine includes a combination of polypeptides including a *P. ariasi* polypeptide having an amino acid sequence as set forth as SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and a *P. perniciosus* polypeptide having an amino acid sequence as set forth as SEQ ID NO:55, SEQ ID NO:63, SEQ ID NO:73, SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and a *P. perniciosus* polypeptide having an amino acid sequence as set forth as SEQ ID NO:55, SEQ ID NO:63, SEQ ID NO:73, SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an

immunogenic fragment thereof, or any combination thereof. In another embodiment, the immunogenic composition or vaccine includes a combination of polypeptides including a *P. ariasi* polypeptide having an amino acid sequence as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and a *P. perniciosus* polypeptide having an amino acid sequence as set forth as SEQ ID NO:55, SEQ ID NO:63, SEQ ID NO:73, SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In yet another embodiment, the immunogenic composition or vaccine includes a combination of polypeptides including a *P. ariasi* polypeptide having an amino acid sequence as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and a *P. perniciosus* polypeptide having an amino acid sequence as set forth as SEQ ID NO:73, SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In yet another embodiment, the immunogenic composition or vaccine includes a combination of polypeptides including a *P. ariasi* polypeptide having an amino acid sequence as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and a *P. perniciosus* polypeptide having an amino acid sequence as set forth as SEQ ID NO:75 a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In a further embodiment, the immunogenic composition or vaccine includes a combination of four *P. ariasi* polypeptides as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and two *P. perniciosus* polypeptides as set forth as SEQ ID NO:73, SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In another embodiment, the immunogenic composition or vaccine includes a combination of four *P. ariasi* polypeptides as set forth as SEQ ID NO:11, SEQ ID

NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and one *P. perniciosus* polypeptide as set forth as SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof.

In one embodiment, the immunogenic composition or the vaccine comprises an effective amount of a recombinant vector expressing at least one *P. ariasi* polypeptide disclosed herein and a pharmaceutically acceptable vehicle or excipient. In one specific, non-limiting example the recombinant vector encodes at least a polypeptide having a sequence as set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, or SEQ ID NO:47, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In another specific, non-limiting example the recombinant vector encodes a polypeptide having a sequence as set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:29, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:39, or SEQ ID NO:43, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In yet another specific, non-limiting example the recombinant vector encodes at least a polypeptide having a sequence as set forth as SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, or SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In yet another specific, non-limiting example the recombinant vector encodes at least a polypeptide having a sequence as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, or SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In a particular embodiment the immunogenic composition or vaccine comprises recombinant vector(s) expressing at least the five polypeptides as set forth as SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:19, SEQ ID

NO:35, and SEQ ID NO:39. In a particular embodiment the immunogenic composition or vaccine comprises recombinant vector(s) expressing at least the four polypeptides as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, and SEQ ID NO:39.

In another embodiment, the immunogenic composition or the vaccine comprises an effective amount of a recombinant vector expressing at least one *P. perniciosus* polypeptide disclosed herein and a pharmaceutically acceptable vehicle or excipient. In one specific, non-limiting example the recombinant vector encodes at least a polypeptide having a sequence as set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In another specific, non-limiting example the recombinant vector encodes at least a polypeptide having a sequence as set forth SEQ ID NO:55, SEQ ID NO:63, SEQ ID NO:73, or SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In yet another specific, non-limiting example the recombinant vector encodes at least a polypeptide having a sequence as set forth as SEQ ID NO:73, or SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In a particular embodiment the immunogenic composition or vaccine comprises recombinant vector(s) expressing at least the four polypeptides as set forth as SEQ ID NO:55, SEQ ID NO:63, SEQ ID NO:73, and SEQ ID NO:75. In a particular embodiment the immunogenic composition or vaccine comprises recombinant vector(s) expressing at least the two polypeptides as set forth as SEQ ID NO:73, and SEQ ID NO:75. In a particular embodiment the immunogenic composition or vaccine comprises a recombinant vector expressing at least the polypeptide as set forth as SEQ ID NO:75.

In a further embodiment, the immunogenic composition or the vaccine comprises a combination including a recombinant vector encoding at least one *P. ariasi* polypeptide disclosed herein and encoding at least one *P. perniciosus*

polypeptide disclosed herein. In one embodiment, the combination includes a recombinant vector encoding at least a *P. ariasi* polypeptide having an amino acid sequence as set forth as SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and at least a *P. perniciosus* polypeptide having an amino acid sequence as set forth as SEQ ID NO:55, SEQ ID NO:63, SEQ ID NO:73, SEQ ID NO:75 a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In another embodiment, the combination includes a recombinant vector encoding at least a *P. ariasi* polypeptide having an amino acid sequence as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and at least a *P. perniciosus* polypeptide having an amino acid sequence as set forth as SEQ ID NO:55, SEQ ID NO:63, SEQ ID NO:73, SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In another embodiment, the combination includes a recombinant vector encoding at least a *P. ariasi* polypeptide having an amino acid sequence as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and at least a *P. perniciosus* polypeptide having an amino acid sequence as set forth as SEQ ID NO:73, SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In yet another embodiment, the combination includes a recombinant vector encoding at least a *P. ariasi* polypeptide having an amino acid sequence as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and at least a *P. perniciosus* polypeptide having an amino acid sequence as set forth as SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In a further embodiment, the combination includes recombinant vector(s) encoding at least four *P. ariasi* polypeptides as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID

NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and at least two *P. perniciosus* polypeptides as set forth as SEQ ID NO:73, SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof. In another embodiment, the combination includes recombinant vector(s) encoding at least four *P. ariasi* polypeptides as set forth as SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:35, SEQ ID NO:39, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and at least the *P. perniciosus* polypeptide as set forth as SEQ ID NO:75, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof.

In one embodiment, the *P. ariasi* polypeptide(s) having an amino acid sequence as set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, or SEQ ID NO:47, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, and the *P. perniciosus* polypeptide(s) having an amino acid sequence as set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, are encoded by the same recombinant vector. In another embodiment, the *P. ariasi* polypeptide(s) having an amino acid sequence as set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, or SEQ ID NO:47, a conservative variant, a fusion protein, a homolog, or an

immunogenic fragment thereof, or any combination thereof, and the *P. perniciosus* polypeptide(s) having an amino acid sequence as set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83, a conservative variant, a fusion protein, a homolog, or an immunogenic fragment thereof, or any combination thereof, are encoded by different recombinant vectors.

The *P. ariasi* polypeptide or a *P. perniciosus* polypeptide can be administered by any means known to one of skill in the art (See Banga, A., "Parenteral Controlled Delivery of Therapeutic Peptides and Proteins," *Therapeutic Peptides and Proteins*, Technomic Publishing Co., Inc., Lancaster, PA, 1995) such as by intramuscular, intradermal, subcutaneous, or intravenous injection, but even oral, nasal, or, anal administration is contemplated. In one embodiment, administration is by subcutaneous, intradermal, or intramuscular injection using a needleless injector (Biojector, Bioject, Oregon, USA).

To extend the time during which the peptide or protein is available to stimulate a response, the peptide or protein can be provided as an implant, an oily injection, or as a particulate system. The particulate system can be a microparticle, a microcapsule, a microsphere, a nanocapsule, or similar particle. (*see, e.g.*, Banja, *supra)*. A particulate carrier based on a synthetic polymer has been shown to act as an adjuvant to enhance the immune response, in addition to providing a controlled release. Aluminum salts may also be used as adjuvants to produce a humoral immune response. Thus, in one embodiment, a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide is administered in a manner to induce a humoral response.

In another embodiment, a *P. ariasi* polypeptide or a *P. perniciosus* polypeptide is administered in a manner to direct the immune response to a cellular response (that is, a CTL response), rather than a humoral (antibody) response. A number of means for inducing cellular responses, both *in vitro* and *in vivo*, are known. Lipids have been identified as agents capable of assisting in priming CTL *in vivo* against various antigens. For example, as described in U.S. Patent No. 5,662,907, palmitic acid residues can be attached to the alpha and epsilon amino

groups of a lysine residue and then linked (*e.g.*, via one or more linking residues, such as glycine, glycine-glycine, serine, serine-serine, or the like) to an immunogenic peptide. The lipidated peptide can then be injected directly in a micellar form, incorporated in a liposome, or emulsified in an adjuvant. As another example, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylseryl-serine can be used to prime tumor specific CTL when covalently attached to an appropriate peptide (*see*, Deres *et al.*, *Nature* **342**:561, 1989). Further, as the induction of neutralizing antibodies can also be primed with the same molecule conjugated to a peptide which displays an appropriate epitope, the two compositions can be combined to elicit both humoral and cell-mediated responses where that is deemed desirable.

In yet another embodiment, an MHC class II-restricted T-helper epitope is added to the polypeptide of the disclosure to induce T-helper cells to secrete cytokines in the microenvironment to activate CTL precursor cells. The technique further involves adding short lipid molecules to retain the construct at the site of the injection for several days to localize the antigen at the site of the injection and enhance its proximity to dendritic cells or other "professional" antigen presenting cells over a period of time (see Chesnut *et al.*, "Design and Testing of Peptide-Based Cytotoxic T-Cell-Mediated Immunotherapeutics to Treat Infectious Diseases and Cancer," Powell, *et al.*, (eds.), *Vaccine Design, the Subunit and Adjuvant Approach*, Plenum Press, New York, 1995).

An immunogenic composition or a vaccine according to the disclosure can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical or veterinary art. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts, taking into consideration such factors as the age, sex, weight, species, and condition of the particular subject, and the route of administration. The immunogenic composition or the vaccine can be administered alone, or in combination with adjuvant(s) and/or with other antigen(s). The other antigen(s) can be a Leishmania antigen. In one embodiment, the Leishmania antigen is the A2 antigen, such as the A2 antigen from *L. infantum* (see PCT Patent Application WO 95/06729 and in particular the sequence given in SEQ ID NO:2). The other

antigen(s) can be present in the composition as a protein, or as an immunological fragment thereof (*e.g.*, an epitope), or as an insert in an expression vector (*e.g.*, recombinant viral vector, recombinant plasmid, in particular the pVR1012 (Vical Inc.; Hartikka J. *et al.*, *Human Gene Therapy* 7:1205-1217, 1996)).

Any immunogenic composition, vaccine, or therapeutic composition according to the disclosure can be mixed with an adjuvant.

Polypeptide-based compositions:

In several embodiments, the polypeptide-based immunogenic compositions and vaccines according to the disclosure are formulated with (1) vitamin E, saponin (*e.g.*, Quil A™, QS21™), aluminum hydroxide, aluminum phosphate, aluminum oxide ("Vaccine Design, The subunit and adjuvant approach," *Pharmaceutical Biotechnology*, vol. 6, Edited by Micheal F. Powell and Mark J. Newman, 1995, Plenum Press New York), (2) an acrylic acid or methacrylic acid polymer, a polymer of maleic anhydride and of alkenyl derivative, (3) an immunostimulating sequence (ISS), in particular an oligodeoxyribonucleotidic sequence bearing one or more non-methylated CpG groups (Klinman D. M. *et al.*, *Proc. Natl. Acad. Sci. USA* 93:2879-2883, 1996; WO 98/16247), (4) to formulate the immunogenic or vaccine preparation in the form of an oil-in-water emulsion, in particular the SPT emulsion described on page 147 of "Vaccine Design, The Subunit and Adjuvant Approach" edited by M. Powell and M. Newman, Plenum Press, 1995, and the emulsion MF59 described on page 183 of this same book, (5) cytokines, or (6) combinations or mixtures thereof.

The cytokine (5) could be added to the composition, such as, but not limited to, GM-CSF or cytokines inducing Th1 (*e.g.*, IL12). All these cytokines can be added to the composition as a protein or as a vector encoding this cytokine protein. In one embodiment, the cytokines are from canine origin, *e.g.*, canine GM-CSF, for which a gene sequence has been deposited at the GenBank database (accession number S49738). This sequence can be used to create the vector in a manner similar to what was made in the PCT Patent Application WO 00/77210.

In one specific, non-limiting example the adjuvant contains two or more of an emulsifier, a micelle-forming agent, or an oil. Suitable emulsifiers, micelle-forming agents, and oils are detailed in U.S. Patent Nos. 5,585,103; 5,709,860;

5,270,202; and 5,695,770. An emulsifier is any molecule that allows the components of the emulsion to remain as a stable emulsion. Such emulsifiers include polysorbate 80 (Sorbitan-mono-9-octadecenoate-poly(oxy-1,2-ethanediyl); manufactured by ICI Americas, Wilmington, Del.), polysorbate 20, polysorbate 21, polysorbate 40, polysorbate 60, polysorbate 61, polysorbate 85, dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, TEEPOL HB7 ™, and SPAN 80 ™ SPAN 85 ™, ethoxylated fatty alcohols, ethoxylated fatty acids, ethoxylated castor oil (hydrogenated or not). In one embodiment, these emulsifiers are provided in an amount of approximately 0.05 to approximately 0.5%. In another embodiment, these emulsifiers are provided in an amount of approximately 0.2%. A micelle forming agent is an agent which is able to stabilize the emulsion formed with the other components such that a micelle-like structure is formed.

Examples of such agents include polymer surfactants described by BASF Wyandotte publications, *e.g.*, Schmolka, *J. Am. Oil. Chem. Soc.* **54**:110, 1977, and Hunter *et al.*, *J. Immunol.* **129**:1244, 1981, PLURONIC™ L62LF, L101, L121, and L64, PEG1000, and TETRONIC™ 1501, 150R1, 701, 901, 1301, and 130R1. The chemical structures of such agents are well known in the art. In one embodiment, the agent is chosen to have a hydrophile-lipophile balance (HLB) of between about 0 and about 2, as defined by Hunter and Bennett, *J. Immun.* **133**:3167, 1984. In one embodiment, the agent can be provided in an effective amount, for example between about 0.5 and about 10%. In another embodiment, the agent can be provided in an effective amount, for example between about 1.25 and about 5%.

In one embodiment, the oil included in the composition is chosen to promote the retention of the antigen in an oil-in-water emulsion, *i.e.*, to provide a vehicle for the desired antigen. In another embodiment, the oil has a melting temperature of less than about 65° C such that emulsion is formed either at room temperature (about 20° C to about 25° C), or once the temperature of the emulsion is brought down to room temperature.

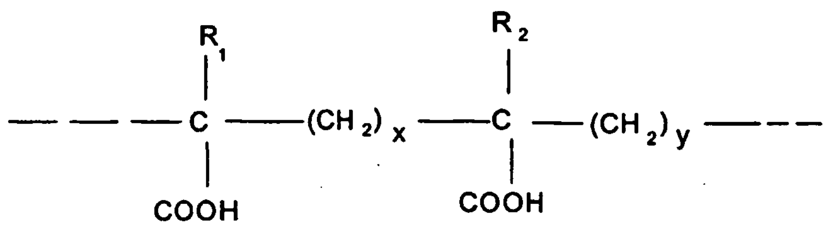
The oil-in-water emulsion (4) can be based in particular on light liquid paraffin oil (European Pharmacopea type); isoprenoid oil such as squalane, squalene, EICOSANE ™ or tetratetracontane; oil resulting from the oligomerization of alkenes, in particular of isobutene or decene; esters of acids or of alcohols

containing a linear alkyl group, more particularly plant oils, ethyl oleate, propylene glycol di(caprylate/caprate), glyceryl tri(caprylate/caprate) or propylene glycol dioleate; esters of branched fatty acids or alcohols, in particular isostearic acid esters. The oil is used in combination with emulsifiers to form the emulsion. In several embodiments, the emulsifiers are nonionic surfactants, in particular esters of sorbitan, mannide (*e.g.*, anhydromannitol oleate), glycerol, polyglycerol, propylene glycol, and oleic, isostearic, ricinoleic, or hydroxystearic acid, which are for example, ethoxylated, and polyoxypropylene-polyoxyethylene copolymer blocks, in particular the Pluronic® products, especially L121. In one specific, non-limiting example, the oil is provided in an amount between about 1 and about 60%. In another specific, non-limiting example, the oil is provided in an amount between about 5 and about 30%. In one embodiment, the adjuvant is a mixture of emulsifiers, micelle-forming agent, and oil available under the name Provax® (IDEC Pharmaceuticals, San Diego, CA).

The acrylic acid or methacrylic acid polymers (2) can be cross-linked in particular with polyalkenyl ethers of sugars or of polyalcohols. These compounds are known under the term "carbomer" (*Pharmeuropa*, Vol. 8, No. 2, June 1996). A person skilled in the art may also refer to U.S. Patent No. 2,909,462 describing such acrylic polymers cross-linked with a polyhydroxylated compound containing at least 3 hydroxyl groups. In one embodiment, a polyhydroxylated compound contains not more than 8 hydroxyl groups. In another embodiment, the hydrogen atoms of at least 3 hydroxyls are replaced with unsaturated aliphatic radicals containing at least 2 carbon atoms. In other embodiments, radicals contain from about 2 to about 4 carbon atoms, *e.g.*, vinyls, allyls, and other ethylenically unsaturated groups. The unsaturated radicals can themselves contain other substituents, such as methyl. The products sold under the name Carbopol® (Noveon Inc., Ohio, USA) are particularly suitable. They are cross-linked with an allyl sucrose or with allylpentaerythritol. Among these, mention may be made of the products Carbopol® 974P, 934P, and 971P.

Among the copolymers of maleic anhydride and of an alkenyl derivative, such as the EMA® products (Monsanto) which are copolymers of maleic anhydride and of ethylene, which may be linear or cross-linked, for example cross-linked with

divinyl ether. Reference may be made to J. Fields *et al.*, *Nature* **186**:778-780, 1960. In one embodiment, the acrylic acid or methacrylic acid polymers and the EMA® products are formed from units based on the following formula:



in which:

- R_1 and R_2, which may be identical or different, represent H or CH_3
- x = 0 or 1, in one embodiment, x = 1
- y = 1 or 2, with x + y = 2.

For the EMA® products, x = 0 and y = 2. For the carbomers, x = y = 1.

In one embodiment, the dissolution of these polymers in water leads to an acid solution, which is neutralized to physiological pH, in order to give the adjuvant solution into which the immunogenic composition or the vaccine itself is incorporated. The carboxyl groups of the polymer are then partly in COO^- form.

In one embodiment, a solution of adjuvant, especially of carbomer, is prepared in distilled water. In another embodiment, a solution of adjuvant, especially of carbomer, is prepared in the presence of sodium chloride, the solution obtained being at acidic pH. In another embodiment, this stock solution is diluted by adding it or a substantial part thereof, to the desired quantity (for obtaining the desired final concentration) of water charged with NaCl. In yet another embodiment, stock solution is diluted by adding it to the desired quantity of physiological saline (NaCl 9 g/l) with concomitant or subsequent neutralization (pH 7.3 to 7.4). In one embodiment, the stock solution is neutralized with NaOH. This solution, at physiological pH, is used as is for mixing with the immunogenic composition or with the vaccine, which may be stored in freeze-dried, liquid or frozen form.

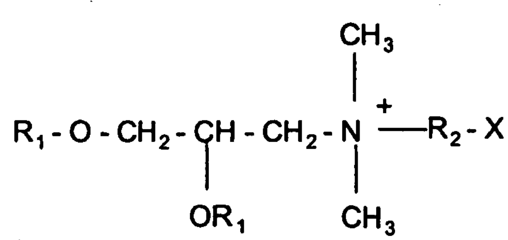
In one embodiment, the polymer concentration in the final vaccine composition is from about 0.01% to about 1.5% weight/volume (W/V). In another embodiment, the final vaccine composition is from about 0.05 to about 1% W/V. In yet another embodiment, the final vaccine composition is from about 0.1 to about 0.4% W/V.

Lipids have been identified as agents capable of stimulating the immune response for various antigens. For example, as described in U.S. Patent No. 5,662,907, palmitic acid residues can be attached to the alpha and epsilon amino groups of a lysine residue and then linked (*e.g.*, via one or more linking residues, such as glycine, glycine-glycine, serine, serine-serine, or the like) to an immunogenic peptide. The lipidated peptide can then be injected directly in a micellar form, incorporated in a liposome, or emulsified in an adjuvant. As another example, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylseryl-serine, can be used.

To extend the time during which the peptide or protein is available to stimulate a response, the peptide or protein can be provided as an implant, an oily injection, or as a particulate system. The particulate system can be a microparticle, a microcapsule, a microsphere, a nanocapsule, or similar particle. (*see, e.g.,* Banja, *supra)*. A particulate excipient based on a synthetic polymer has been shown to act as an adjuvant to enhance the immune response, in addition to providing a controlled release.

Plasmid-based compositions:

In one embodiment, the plasmid-based compositions is formulated with cationic lipids, in particular with cationic lipids containing a quaternary ammonium salt having the following formula:



in which R1 is a saturated or unsaturated linear aliphatic radical from 12 to 18 carbon atoms, R2 is another aliphatic radical comprising from 2 to 3 carbon atoms, and X is an hydroxyl or amine group.

In one embodiment, DMRIE (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanammonium; WO 96/34109) is the cationic lipid. In another embodiment, the cationic lipid is in association with a neutral lipid, for example DOPE (dioleoyl-phosphatidyl-ethanolamine; Behr J. P., *Bioconjugate Chemistry* 5:382-389, 1994), in order to form the DMRIE-DOPE. In yet another embodiment, the mixture is made extemporaneously about 10 minutes to about 60 minutes before administration. In another embodiment, the mixture is made extemporaneously about 30 minutes before administration. In one embodiment, the molar ratio of DMRIE/DOPE is from about 95/5 to about 5/95. In another embodiment, the molar ratio of DMRIE/DOPE is about 1/1. In one embodiment, the weight ratio of plasmid/DMRIE or of DMRIE-DOPE adjuvant is from about 50/1 to about 1/10. In another embodiment, the weight ratio of plasmid/DMRIE or of DMRIE-DOPE adjuvant is from about 10/1 to about 1/5. In yet another embodiment, the weight ratio of plasmid/DMRIE or of DMRIE-DOPE adjuvant is from about 1/1 to about 1/2.

In one embodiment, a cytokine or non-methylated CpG groups is added to the composition, as described above for polypeptide-based compositions. The addition can be done advantageously by a plasmid encoding the cytokine.

Viral vector-based composition:

The recombinant viral vector-based composition can be supplemented with fMLP (N-formyl-methionyl-leucyl-phenylalanine; U.S. Patent No.: 6,017,537) and/or acrylic acid or methacrylic acid polymer adjuvant as described above for polypeptide-based compositions. They can also be formaluted with emulsions as described above.

In one embodiment, cytokines, non-methylated CpG groups, or emulsions are added to the composition as described above for polypeptide-based compositions. The addition can be done advantageously by a viral vector encoding said cytokine.

The immunogenic compositions and vaccines according to the disclosure are conserved and stored either in formulated form at 5°C, or in lyophilized form. In one embodiment, the immunogenic compositions and vaccines according to the disclosure are conserved and stored either in formulated form at 5°C, or in lyophilized form with a stabilizer. Freeze-drying can be done according to well-known standard freeze-drying procedures. The pharmaceutically acceptable stabilizers may be SPGA (sucrose phosphate glutamate albumin) (Bovarnik *et al., J. Bacteriology* **59**:509, 1950), carbohydrates (*e.g.*, sorbitol, mannitol, lactose, sucrose, glucose, dextran, trehalose), sodium glutamate (Tsvetkov T *et al., Cryobiology* **20(3)**:318-23, 1983 ; Israeli E *et al., Cryobiology* **30(5)**:519-23, 1993), proteins such as peptone, albumin, or casein, protein containing agents such as skimmed milk (Mills CK *et al., Cryobiology* **25(2)**:148-52, 1988; Wolff E *et al., Cryobiology* **27(5)**:569-75, 1990), and buffers (*e.g.*, phosphate buffer, alkaline metal phosphate buffer). An adjuvant may be used to make soluble the freeze-dried preparations.

Methods of Immunization

The present disclosure provides methods for inducing an immune response to a Phlebotomus polypeptide in a subject. The present disclosure provides further methods for inhibiting or preventing leishmaniasis in a subject.

These methods include the administration of at least one immunogenic composition or vaccine according to the disclosure.

An immunogenic composition or a vaccine according to the disclosure can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical or veterinary art. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts, taking into consideration such factors as the age, sex, weight, species, and condition of the particular subject, and the route of administration.

If more than one administration is required, they can be administered concurrently (*e.g.*, different compositions given during the same period of time via the same or different routes, or a same composition given in the same period of time via different routes), or sequentially (*e.g.*, the same or different compositions given at least two times via the same or different routes). In one embodiment, the delay

between two sequential administrations is from about 1 week to about 6 months. In another embodiment, the delay is from about 3 weeks to about 6 weeks. In yet another embodiment, the delay is from about 4 weeks. Following vaccination, annual boost administrations may be done. In a prime-boost vaccination schedule advantageously, at least one primo-administration can be done with a composition containing a plasmid according to the disclosure, following by at least one booster administration done with a composition containing a recombinant viral vector according to the disclosure, on the condition that a same Phlebotomus salivary polypeptide is present twice, coded by the plasmid and by the viral vector. Alternatively, the booster administration can be done with a composition containing a polypeptide according to the disclosure, on the condition that a same Phlebotomus salivary polypeptide is present twice, coded by the primo-administration plasmid and in the booster polypeptide-based composition.

In such compositions the antigen(s) may be in admixture with a suitable vehicle or excipient such as sterile water, physiological saline, glucose, or the like. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, adjuvants, gelling, or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as Remington's Pharmaceutical Science, 17th edition, 1985, may be consulted to prepare suitable preparations, without undue experimentation. The compositions can also be lyophilized.

Suitable dosages can also be based upon the examples below. For polypeptide-based compositions, the route of administration can be intradermal (ID), intramuscular (IM), or subcutaneous (SC), intravenous, oral, nasal, or anal. This administration can be made with a syringe and a needle or with a needle-less apparatus like, for example, Biojector (Bioject, Oregon, USA). In several embodiments, polypeptide dosages can be from about 1 to 250 μg/ml, from about 15 to about 150 μg/dose, or from about 20 to about 100 μg/dose. In another embodiment, using a needle-less apparatus, the volume of a dose can be between about 0.1 ml and about 0.5 ml. In yet another embodiment, using a needle-less apparatus, the volume of a dose can be about 0.25 ml. Administration with multiple

points of injection is preferred. In one embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.1 to about 2 ml. In another embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.5 to about 1 ml.

For plasmid-based compositions, the route of administration can be ID, IM, SC, intravenous, oral, nasal, or anal. This administration can be made with a syringe and a needle or with a needle-less apparatus like, for example, Biojector. The dosage is from about 50 μg to about 500 μg per plasmid. When DMRIE-DOPE is added, about 100 μg per plasmid is preferred. In one embodiment, when canine GM-CSF or other cytokine is used, the plasmid encoding this protein is present at a dosage from about 200 μg to about 500 μg. In another embodiment, the plasmid encoding this protein is present at a dosage of about 200 μg. In one embodiment, using a needle-less apparatus, the volume of a dose can be between about 0.1 ml and about 0.5 ml. In another embodiment, the volume of a dose can be about 0.25 ml. In yet another embodiment, administration is performed using multiple points of injection. In one embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.1 to about 2. In another embodiment, the volumes are from about 0.5 to about 1 ml. The dosages are the same as mentioned above.

For recombinant viral vector-based compositions, the route of administration can be ID, IM, SC, intravenous, oral, nasal, or anal. This administration can be made with a syringe and a needle or with a needle-less apparatus like, for example, Biojector. The dosage is from about 10^3 pfu to about 10^9 pfu per recombinant poxvirus vector. In one embodiment, when the vector is a canarypox virus, the dosage is from about 10^5 pfu to about 10^9 pfu. In another embodiment, when the vector is a canarypox virus, the dosage is from about 10^6 pfu to about 10^8 pfu. In one embodiment, the volume of needle-less apparatus doses is between about 0.1 ml and about 0.5 ml. In another embodiment, the volume of needle-less apparatus dose is 0.25 ml. In yet another embodiment, administration is performed using multiple points of injection. In one embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.1 to about 2. In another embodiment, the volumes are from about 0.5 to about 1 ml. The dosages are the same as

mentioned above. In one embodiment, when a syringe with a needle is used, the injection is IM.

In one embodiment, for the prime-boost administration regimen, the prime-administration is made with a plasmid-based composition and the boost administration is made with a recombinant viral vector-based composition. In one embodiment, the boost administration is made with a canarypox vector. Both priming and boosting administrations include vectors encoding at least one identical Phlebotomus salivary antigen, and in one specific, non-limiting example, a Leishmania A2 antigen. The dosage of plasmids and recombinant viral vectors are the same as described above. In one embodiment, the boost administration is done with a polypeptide-based composition. In specific, non-limiting examples, the dosage of polypeptide is from about 1 to about 250 μg/ml, from about 15 to about 150 μg/dose, or from about 20 to about 100 μg/dose.

Immunization by nucleic acid constructs is well known in the art and taught, for example, in U.S. Patent No. 5,643,578 (which describes methods of immunizing vertebrates by introducing DNA encoding a desired antigen to elicit a cell-mediated or a humoral response) and U.S. Patent Nos. 5,593,972 and 5,817,637 (which describe operably linking a nucleic acid sequence encoding an antigen to regulatory sequences enabling expression). U.S. Patent No. 5,880,103 describes several methods of delivery of nucleic acids encoding immunogenic peptides or other antigens to an organism. The methods include liposomal delivery of the nucleic acids (or of the synthetic peptides themselves), and immune-stimulating constructs, or ISCOMS™, negatively charged cage-like structures of 30-40 nm in size formed spontaneously on mixing cholesterol and Quil A™ (saponin). Protective immunity has been generated in a variety of experimental models of infection, including toxoplasmosis and Epstein-Barr virus-induced tumors, using ISCOMS™ as the delivery vehicle for antigens (Mowat and Donachie, *Immunol. Today* **12**:383, 1991). Doses of antigen as low as 1 μg encapsulated in ISCOMS™ have been found to produce class I mediated CTL responses (Takahashi *et al.*, *Nature* **344**:873, 1990).

In another approach to using nucleic acids for immunization, a *P. ariasi* or a *P. perniciosus* polypeptide, or an immunogenic peptide thereof, can also be expressed by attenuated viral hosts or vectors or bacterial vectors. Recombinant

vaccinia virus, adeno-associated virus (AAV), herpes virus, retrovirus, or other viral vectors can be used to express the peptide or protein, thereby eliciting a CTL response. For example, vaccinia vectors and methods useful in immunization protocols are described in U.S. Patent No. 4,722,848. BCG (Bacillus Calmette Guerin) provides another vector for expression of the peptides (see Stover, *Nature* **351**:456-460, 1991).

In one embodiment, a nucleic acid encoding a *P. ariasi* or a *P. perniciosus* polypeptide, or an immunogenic fragment thereof, is introduced directly into cells. For example, the nucleic acid may be loaded onto gold microspheres by standard methods and introduced into the skin by a device such as Bio-Rad's Helios™ Gene Gun. A needless injector can also be utilized, such as a Bioinjector2000™. The nucleic acids can be "naked," consisting of plasmids under control of a strong promoter. Typically, the DNA is injected into muscle, although it can also be injected directly into other sites. Exemplary dosages for injection are around 0.5 μg/kg to about 50 mg/kg, and typically are about 0.005 mg/kg to about 5 mg/kg (see, *e.g.*, U.S. Patent No. 5,589,466). In one embodiment, a prime-boost strategy for immunization is utilized. Thus, in one embodiment, a nucleic acid encoding a *P. ariasi* or a *P. perniciosus* polypeptide is administered to the subject, followed by immunization with an attenuated or inactivated form of Leishmania.

The immunogenic compositions and the vaccines disclosed herein can be administered for preventative and therapeutic treatments. In therapeutic applications, compositions are administered to a subject suffering from a disease, such as *Leishmaniasis*, in a therapeutically effective amount, which is an amount sufficient to cure or at least partially arrest the disease or a sign or symptom of the disease. Amounts effective for this use will depend upon the severity of the disease and the general state of the subject's health. An effective amount of the compound is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer.

Single or multiple administrations of the compositions are administered depending on the dosage and frequency as required and tolerated by the subject. In one embodiment, the dosage is administered once as a bolus, but in another embodiment can be applied periodically until a therapeutic result is achieved.

Generally, the dose is sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity in the subject.

As noted above, the dosage of the composition varies depending on the weight, age, sex, and method of administration. The dosage can also be adjusted by the individual physician as called for based on the particular circumstances. The compositions can be administered conventionally as vaccines containing the active composition as a predetermined quantity of active material calculated to produce the desired therapeutic or immunologic effect in association with the required pharmaceutically acceptable carrier or diluent (*i.e.*, carrier or vehicle). For example, about 50 μg of a DNA construct vaccine of the present disclosure can be injected intradermally three times at two week intervals to produce the desired therapeutic or immunologic effect. In another embodiment, about 1 mg/Kg dosage of a protein vaccine of the present disclosure can be injected intradermally three times at two week intervals to produce the desired therapeutic or immunologic effect.

A vaccine is provided herein that includes a *P. ariasi* or *P. perniciosus* polypeptide or polynucleotide. Administration of the vaccine to a subject, such as a human or veterinary subject, results in resistance to infection with *Leishamania*. In one embodiment, the subject is a human subject. In another embodiment, the subject is a canine subject, such as a dog.

The disclosure is illustrated by the following non-limiting Examples.

EXAMPLES

Example 1
Library Construction

Sand Flies and Preparation of salivary gland homogenate (SGH). Salivary extracts are prepared directly from sand flies captured in the wild in the Cevennes in Southern France (close to Vallerauge (Gard)) which is known to be a valid biotope for the *P. ariasi* sand fly species. Unfed females are captured by aspiration immediately after they land on the outside walls of a tent in which a dog has been placed. Captures are done at the end of July, shortly after dusk on dry days and in

the absence of wind. Outside temperatures should be between 20 and 25°C. Alternatively, salivary extracts of *P. perniciosus* are prepared directly from sand flies captured in the wild in Southern France (near the city of Marseille) which is known to be a valid biotope for the *P. perniciosus* sand fly species. The identification of the sand fly specimen is performed by the visual observation (20x microscope) of the morphology of the spermatheca after dissection, as described in Leger *et al. Ann. Parasitol. Hum. Comp.* **t58(6)**:611-623, 1983).

Salivary glands dissected under a dissection microscope and collected in microfuge tubes in sterile phosphate saline buffer, pH 7.0, are stored in dry ice and transferred to –70 °C until use.

The salivary gland of *P. ariasi* is a sac-like structure consisting of a unicellular epithelium layer surrounding a large lumen (Adler and Theodor, *Ann. Trop. Med. Parasitol.* 20:109, 1926). After a blood meal, the gland total protein content decreases to half or less from its ~1 μg value (Ribeiro *et al., Insect Biochem.* 19:409-412, 1989). Thus, most of the protein from the fly SGH must be destined for secretion. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of SGH reveals a low complexity composition consisting of ~12 major bands varying from 10-100 kDa (Valenzuela *et al., J. Exp. Med.*194:331-42, 2001). For SDS-PAGE, Tris-glycine gels (16%), 1 mm thick, or NUPAGE 12% BIS-tris gels were used (Invitrogen). Gels were run with either Tris-glycine or MOPS Nupage running buffer according to the manufacturer's instructions. To estimate the molecular weight of the samples, See BlueJ markers from Invitrogen (myosin, BSA, glutamic dehydrogenase, alcohol dehydrogenase, carbonic anhydrase, myoglobin, lysozyme, aprotinin, and insulin, chain B) were used. SGH were treated with equal parts of 2X SDS sample buffer (8% SDS in Tris-HCl buffer, 0.5M, pH 6.8, 10% glycerol and 1% bromophenol blue dye). Thirty pairs of homogenized salivary glands per lane (approximately 30μg protein) were applied when visualization of the protein bands by Coomassie blue staining was desired. For amino terminal sequencing of the salivary proteins, 40 homogenized pairs of glands were electrophoresed and transferred to polyvinylidene difluoride (PVDF) membrane using 10 mM CAPS, pH 11, 10% methanol as the transfer buffer on a Blot-Module for the XCell II Mini-Cell (Invitrogen). The membrane was stained with Coomassie

blue in the absence of acetic acid. Stained bands were cut from the PVDF membrane and subjected to Edman degradation using a Procise sequencer (Perkin-Elmer Corp.).

Salivary Gland cDNA Library Construction. P. ariasi salivary gland mRNA was isolated from 100 salivary gland pairs from adult females. The Micro-FastTrack mRNA isolation kit (Invitrogen, San Diego, CA) was used, yielding approximately 100 ng poly (A)+ mRNA. The PCR-based cDNA library was made following the instructions for the SMART cDNA library construction kit (Clontech, Palo Alto, CA). One hundred nanograms of *P. ariasi* salivary gland mRNA was reverse transcribed to cDNA using Superscript II RNase H- reverse transcriptase (Gibco-BRL, Gaithersburg, MD) and the CDS/3' primer (Clontech, Palo Alto, CA) for 1 hour at 42°C. Second strand synthesis was performed using a PCR-based protocol by using the SMART III primer (Clontech, Palo Alto, CA) as the sense primer, and the CDS/3' primer as anti-sense primer. These two primers, additionally, create at the ends of the nascent cDNA, SfiI A and B sites respectively. Double strand cDNA synthesis was done on a Perkin Elmer 9700 Thermal cycler (Perkin Elmer Corp., Foster City, CA) using the Advantage Klen-Taq DNA polymerase (Clontech, Palo Alto, CA). PCR conditions were the following: 94° C for 2 minutes; 19 cycles of 94° C for 10 seconds and 68° C for 6 minutes. Double stranded cDNA was immediately treated with proteinase K (0.8 μg/μl) for 20 minutes at 45° C and washed three times with water using Amicon filters with a 100 kD cut off (Millipore Corp., Bedford MA). The double stranded cDNA was then digested with Sfi I for 2 hours at 50° C (The Sfi I sites were inserted to the cDNA during the second strand synthesis using the SMART III and the CDS/3' primer). The cDNA was then fractionated using columns provided by the manufacturer (Clontech, Palo Alto, CA). Fractions containing cDNA of more than 400 bp were pooled, concentrated, and washed three times with water using an Amicon filter with a 100 kDa cut-off. The cDNA was concentrated to a volume of 7 μl. The concentrated cDNA was then ligated into a lambda triplex2 vector (Clontech, Palo Alto, CA), and the resulting ligation reaction was packed using the Gigapack gold III from Stratagene/Biocrest (Cedar Creek, TE) following manufacturer's specifications. The obtained library was plated by infecting log phase XL1- blue

cells (Clontech, Palo Alto, CA) and the amount of recombinants was determined by PCR using vector primers flanking the inserted cDNA and visualized on a 1.1 % agarose gel with ethidium bromide (1.5 μg/ml)

Massive Sequencing of P. ariasi Salivary Gland cDNA Library. P. ariasi salivary gland cDNA library was plated to approximately 200 plaques per plate (150 mm Petri dish). The plaques were randomly picked and transferred to a 96 well polypropylene plate containing 100 μl of water per well. The plate was covered and placed on a gyrator shaker for 1 hour at room temperature. Four microliters of a phage sample was used as a template for a PCR reaction to amplify random cDNAs. The primers used for this reaction were sequences from the triplex2 vector, the primers were named PT2F1 (5'- AAGTACTCT AGCAAT TGTGAGC- 3') (SEQ ID NO:85) which is positioned upstream of the cDNA of interest (5' end), and PT2R1 (5'- CTCTTCGCTATTACGCCAGCT G- 3') (SEQ ID NO:86) which is positioned downstream of the cDNA of interest (3' end). Platinum Taq polymerase (Gibco-BRL, Gaithersburg, MD) was used for these reactions. Amplification conditions were: 1 hold of 75° C for 3 minutes, 1 hold of 94° C for 3 minutes, and 34 cycles of 94° C for 30 seconds, 49° C for 30 seconds and 72° C for 1 minute and 20 seconds. Amplified products were visualized on a 1.1% agarose gel with ethidium bromide. Clean PCR was used as a template for a cycle sequencing reaction using the DTCS labeling kit from Beckman Coulter Inc. (Fullerton, CA). The primer used for sequencing (PT2F3) (5'-TCTCGGGAAGCGCGCCATTGTGTT - 3') (SEQ ID NO:87) is upstream of the inserted cDNA and downstream of the primer PT2F1. Sequencing reaction was performed on a Perkin Elmer 9700 thermacycler. Conditions were 75°C for 2 minutes, 94°C for 4 minutes, and 30 cycles of 96°C for 20 seconds, 50°C for 20 seconds and 60°C for 4 minutes.

After cycle sequencing the samples, a cleaning step was done using the multi-screen 96 well plate cleaning system from Millipore (Bedford, MA). The 96 well multi-screening plate was prepared by adding a fixed amount (according to the manufacturer's specifications) of Sephadex-50 (Amersham Pharmacia Biotech, Piscataway, NJ) and 300 μl of deionized water. After 1 hour of incubation at room temperature, the water was removed from the multi screen plate by centrifugation at

750 g for 5 minutes. After the Sephadex in the multi-screen plate was partially dried, the whole cycle sequencing reaction was added to the center of each well, centrifuged at 750 g for 5 minutes and the clean sample was collected on a sequencing microtiter plate (Beckman Coulter, Fullerton, CA). The plate was then dried on Speed-Vac SC 110 model with a microtiter plate holder (Savant Instruments Inc, Holbrook, NY). The dried samples were immediately resuspended with 25 µl of deionized ultrapure formamide (J.T. Baker, Phillipsburg, NJ), and one drop of mineral oil was added to the top of each sample. Samples were sequenced immediately on a CEQ 2000 DNA sequencing instrument (Beckman Coulter Inc., Fullerton, CA) or stored at -30°C. The entire cDNA of selected genes was fully sequenced using custom primers using a CEQ 2000 DNA sequencing instrument (Beckman Coulter Inc., Fullerton, CA), as described above.

Accordingly, a cDNA library was also constructed with *P. perniciosus* salivary glands and sequenced.

DNA Vaccine Construction and Description of the VR1020 Vector. The genes coding for the predicted secreted proteins were amplified from *P. ariasi* specific cDNA and from *P. perniciosus* specific cDNA by PCR using Platinum *Taq* polymerase (GIBCO BRL) and specific primers carrying the Predicted N-terminus (Forward primer); and the stop codon (Reverse primer) of the selected cDNA.

The PCR product was immediately cloned into the custom made VR-2001-TOPO (derived fromVR1020 vector) cloning vector following the manufacturer's specifications (Invitrogen). The ligation mixture was used to transform TOP10 cells (Invitrogen) and the cells were incubated overnight at 37° C. Eight colonies were picked and mixed with 10 µl of sterile water. Five µl of each sample were transferred to Luria broth (LB) with ampicillin (100 µg/ml) and grown at 37° C. The other 5 µl were used as a template for a PCR reaction using two vector-specific primers from the PCRII vector to confirm the presence of the insert and for sequencing analysis. After visualization of the PCR product on a 1.1% agarose gel, the eight PCR products were completely sequenced as described above using a CEQ2000 DNA sequencing instrument (Beckman Coulter). Cells containing the plasmid carrying the selected *P. ariasi* gene were grown overnight at 37° C on Luria broth with ampicillin (100 µg/ml), and plasmid isolation was performed using the

Wizard Miniprep kit (Promega). The VR-2001-TOPO (a variant of the VR1020 plasmid from Vical) plasmid contains a kanamycin resistance gene, the human cytomegalovirus promoter, and the tissue plasminogen activator signal peptide upstream of the TOPO TA cloning site. The sample that contained the sequence from the start codon to the stop codon in the right orientation and in the correct open-reading-frame following the nucleotide sequence encoding the tissue plasminogen activator signal peptide was chosen.

Plasmids were transformed into the SCS-1 strain of *E. coli* (Stratagene, La Jolla, CA) according to the manufacturer's instructions. The transformed bacteria were grown in LB medium and the plasmid was subsequently purified using a commercial plasmid purification kit (Qiagen, Courtaboeuf, France). Individual plasmids were quality controlled for identity based on a restriction profile.

These plasmids were named and encoded the following proteins:

Table 1

Plasmid name	Protein encoded
PJV001	PRL-P4-A10
PJV002	PRL-P4-D6
PJV003	PRL-P4-E5
PJV004	PRL-P4-G7
PJV005	PRM-P5-D6
PJV006	PRM-P5-E9
PJV007	PRM-P5-F12
PJV008	PRM-P5-F2
PJV009	PRM-P5-G11
PJV010	PRM-P5-H4
PJV011	PRS-P1-B11
PJV012	PRS-P1-B4
PJV013	PRS-P1-E7
PJV014	PRS-P1-G9
PJV015	PRS-P2-C8
PJV016	PRS-P2-G8
PJV017	PRL-P4-A9
PJV018	PRL-P4-C10
PJV019	PRL-P4-D7
PJV020	PRL-P4-F3
PJV021	PRL-P4-G12

PJV022	PRL-P6-E11
PJV023	PRM-P3-A6
PJV024	PRM-P3-F11
PJV031	PERL-P7-G8
PJV032	PERL-P6-H9
PJV033	PERL-P7-C2
PJV034	PERL-P6-H1
PJV035	PERL-P3-E11
PJV036	PERL-P7-G12
PJV037	PERL-P3-C9
PVJ038	PERM-P2-A10
PVJ039	PERL-P6-H11
PJV040	PERS-P1-H11
PJV041	PERM-P2-G11
PJV042	PERM-P5-E2
PJV025	PERM-P5-C11
PJV026	PERM-P5-H8
PJV027	PERL-P3-B3
PJV028	PERM-P2-D11
PJV029	PERM-P5-E3
PJV030	PERM-P2-F11

Example 2

DNA and Predicted Protein Sequence Analysis.

DNA data derived from the mass sequencing project were analyzed by an in-house program written in VisualBASIC (Microsoft). This program removed vector and primer sequences from the raw sequence. Stripped sequences were compared to the NCBI non-redundant protein database using the program BlastX using the BLOSUM-62 matrix (Altschul *et al. Nucleic Acids Research* **25**:3389, 1997). DNA sequences were clustered by blasting the database against itself with a preselected threshold cutoff, usually $1e^{-10}$ (BlastN program) (Altschul *et al. Nucleic Acids Research* **25**:3389, 1997). Sequences from the same cluster were aligned using ClustalX (Jeanmougin *et al., Trends Biochem. Sci.* 23:403, 1998). To find the cDNA sequences corresponding to the amino acid sequence obtained by Edman degradation of the proteins transferred to PVDF membranes from SDS-PAGE gels, a search program was written that checked these amino acid sequences against the three possible protein translations of each cDNA sequence obtained in the mass

sequencing project. This was written using the same approach used in the BLOCKS (Henikoff *et al., Bioinformatics* 15:471, 1999) or Prosite (Bairoch, *Nucleic Acids Res.* 19(Suppl.):2241,1991) programs. Protein translations of the full-length clones were further processed to identify the predicted signal peptides using the Signal P program (Nielsen *et al., Protein Eng.* 10:1, 1997), available online. Predicted signal peptide cleaved sites were compared to the N-terminus sequence obtained from Edman degradation of *Phlebotomus* salivary proteins. Estimation of isoelectric point and molecular weight of translated protein was performed using the DNA STAR program (DNASTAR). Full-length translated protein sequence information was compared with the non-redundant protein database of NCBI using the BLAST-P program (Altschul *et al. Nucleic Acids Research* **25**:3389, 1997) and searched for motifs by submitting each sequence to the electronic database.

To characterize the primary structure of the main proteins of *P. ariasi* and *P. perniciosus* SGH, SDS-PAGE gels were transferred to PVDF membranes, and the amino terminal sequence of each cut band by Edman degradation was estimated.

Example 3

DNA Vaccination in Mice

For genetic immunization, Swiss Webster mice were purchased from Taconic Farms. Mice were maintained in the NIAID Animal Care Facility under pathogen-free conditions. Mice were inoculated in the right ear with 30 μg of the plasmid encoding the selected cDNA from *P. ariasi* suspended in 5 μl of PBS. Each group was boosted 2 wk later using the same regimen. Mice were challenged on the opposite ear with salivary gland homogenate of *P. ariasi* and delayed type hypersensitivity (DTH) response was measured 24 hours after the injection by measuring thickness and redness of ear (++: at least 2 mice with a good DTH response, +++ : at least three mice had a good DTH response, Table 2).

Table 2

Plasmid name	DTH response
PJV002	-
PJV016	-
PJV008	-
PJV017	-
PJV021	+++
PJV013	-
PJV024	+++
PJV022	+++
PJV007	-
PJV005	+++
PJV009	+++
PJV023	++
PJV010	-
PJV012	+++
PJV003	-
PJV014	-
PJV015	++
PJV019	-
PJV018	-
PJV011	-
PJV020	+++
PJV001	+++
PJV006	+++
PJV004	++

Example 4

Production of an Immune Response in Dogs

In a first experiment DTH (delayed type hypersensitivity) reaction was performed in dogs with natural immunity against the leishmaniasis in order to determine which *P. ariasi* salivary proteins are recognized by a protective immune response. Dogs with natural immunity survived without symptoms after two years of exposure in an endemic area. In a second experiment, naïve dogs were immunized with the 24 *P. ariasi* salivary gland proteins expressed by a plasmid in order to evaluate the capability to induce a cellular immune response measured by DTH.

Twelve dogs approximately three years old with natural immunity against Leishmaniasis were injected, via an intradermal route (ID) in the back after shaving, with 100μg of each individual plasmid suspended in 100μl of PBS. Each plasmid

was injected at a different point. The points were separated by at least 3 cm to avoid interference between DTH responses. The negative control (100µl of buffer) was also inoculated by ID route.

The DTH response was assessed 72 hours after injection by measuring the larger diameter of the skin tumefaction area (see Table 3). The results are expressed as the mean value of the tumefaction area for all the dogs and as a percentage of dogs having a positive DTH response. A positive DTH is a tumefaction area diameter greater than or equal to 4 mm at 72 hours after injection.

Table 3

Plasmids	Mean diameter of the tumefaction area (mm)	Percentage of dogs with a tumefaction diameter ≥ 4mm
PJV018	1.1	18%
PJV016	1.4	20%
PJV005	2.0	27%
PJV006	1.4	27%
PJV008	2.0	27%
PJV011	1.6	27%
PJV013	1.7	27%
PJV020	1.4	27%
PJV023	1.5	27%
PJV015	2.4	36%
PJV024	2.1	36%
PJV004	2.3	40%
PJV009	2.9	46%
PJV014	2.7	46%
PJV019	2.9	46%
PJV002	2.8	46%
PJV021	3.3	55%
PJV007	3.3	55%
PJV017	3.9	58%
PJV001	4.4	64%
PJV022	4.1	64%
PJV003	6.0	82%
PJV012	6.1	91%
PJV010	6.3	100%

The data in Table 3 can be divided into three groups: a first group corresponding to plasmids number PJV001, PJV022, PJV003, PJV012, and PJV010 showing a strong DTH response, a second group corresponding to PJV018, PJV016, PJV005, PJV006, PJV008, PJV011, PJV013, PJV020, PJV023, PJV015, and PJV024 showing a low DTH response, and a last group (with PJV004, PJV009, PJV014, PJV019, PJV002, PJV021, PJV007, and PJV017) showing an intermediate DTH response.

In a second study, 10 naïve dogs 4 to 6 months old were immunized by ID injection in 10 points (100µl per point) in the right ear with a pool of the 24 plasmids (PJV001 to PJV024), 100µg for each one suspended in 1000µl of PBS. On day 21, dogs were injected in 10 points (100µl per point) in the left ear and in 10 points (100µl per point) in the belly with a pool of the 24 plasmids, 100µg for each one suspended in 2000µl of PBS. All dogs were challenged on day 35 by inoculation by ID route in the back (after shaving), with 100µg of each individual plasmid suspended in 100µl of PBS. Each plasmid was injected at a different point. The points were separated by at least 3 cm to avoid interference. As a negative control, 100µl of buffer was inoculated intradermally. The DTH response was assessed 72 hours after challenge, by measuring the larger diameter of the skin tumefaction area (see Table 4). The results are expressed as the mean value of the tumefaction area for all the dogs and as a percentage of dogs having a positive DTH response. A positive DTH is a tumefaction area diameter higher or equal of 4 mm at 72 hours after injection.

Table 4

Plasmids	Mean diameter of the tumefaction area (mm)	Percentage of dogs with a tumefaction diameter ≥4mm
PJV018	4.2	60%
PJV016	3.2	56%
PJV005	3.9	60%
PJV006	3.3	50%
PJV008	3.9	70%
PJV011	4.9	89%
PJV013	3.2	56%
PJV020	2.5	50%
PJV023	3.9	67%
PJV015	2.7	44%
PJV024	5.3	78%
PJV004	3.8	56%
PJV009	3.7	70%
PJV014	2.9	44%
PJV019	1.8	33%
PJV002	2.7	50%
PJV021	4.2	70%
PJV007	3.5	70%
PJV017	3.1	56%
PJV001	2.1	40%
PJV022	6.4	100%
PJV003	4.3	70%
PJV012	3.2	60%
PJV010	2.4	40%

The results of this table show that all plasmids can induce cellular immunity in dogs after injection, as revealed by a DTH response. The variation of the DTH response level may be due to the variation of the expression of the insert.

It will be apparent that the precise details of the methods described may be varied or modified without departing from the spirit of the described disclosure. We claim all such modifications and variations that fall within the scope and spirit of the claims below.